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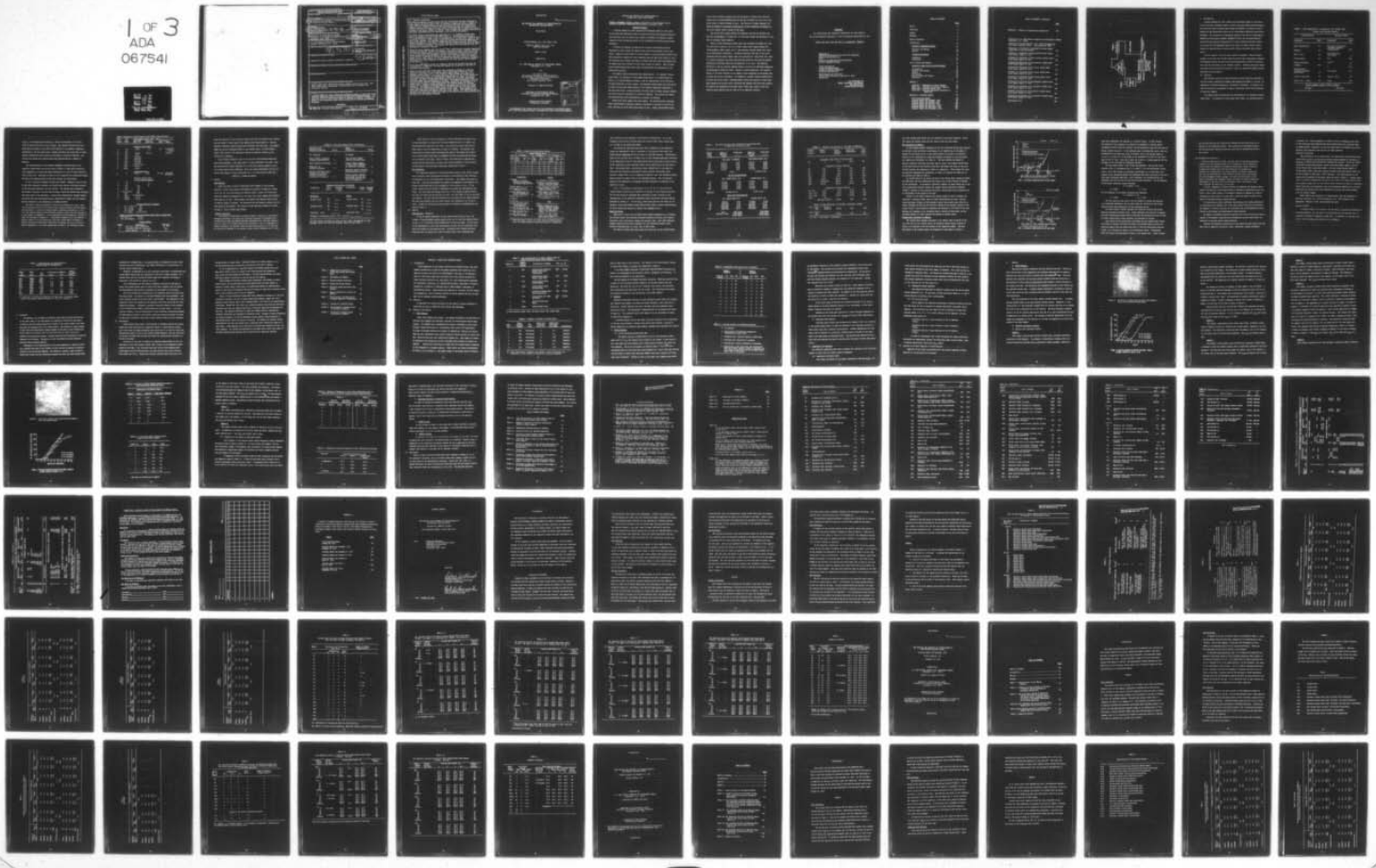

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20. Abstract (continued)

A process of membrane ultrafiltration and post chlorination has been developed to treat laundry and shower waste waters for potential recycle and reuse. Samples of water taken at various stages in the purification process have been evaluated in respect to their immediate toxicity when given orally to mice, as a primary skin irritant when kept in contact with the intact and abraded skin of rabbits and as an irritant to the ocular tissues in rabbits. Each of these tests provided quantitative data which correlated with the efficiency of the purification process.

The sample waters investigated were identified as: (1) synthetic laundry waste water, (2) synthetic or real shower waste water, (3) ultrafiltrates of Group 1, (4) ultrafiltrates of Group 2, and (5) concentrates (10 to 1000 fold) of waters from each of the 4 groups. The synthetic waste waters were constituted by adding to tap water known amounts of the chemical components identified in the real waste water, thereby providing for study water of known chemical composition which could be duplicated or altered as required. The various real wastes and ultrafiltrates were concentrated by a process of freezing.

Ninety-five water samples have been tested. The ultrafiltrates (including those concentrated by freezing) caused no irritation in the skin or eye of rabbits, and were not toxic when given orally to mice. Highly concentrated wastes led to skin irritation ranging from mild erythema to necrosis and corrosion; evoked mild to severe conjunctivitis in the eye of rabbits and were toxic when given orally in massive dosage to mice. The severity of these responses was directly related to increasing concentration of waste chemicals as measured by the total organic carbon content of the water.

Ten of the water samples tested for mutagenic activity by the Ames test were all non-mutagenic; of these ten, six have been tested for irritation to the skin of volunteer human subjects.

Using a 21-day Continuous closed patch test technique in humans as a test for cumulative irritancy, two out of eight tested water samples namely S40 (100X synthetic shower waste) and L7 (50X synthetic laundry waste) were found to be significantly irritating. Irritation resulting from any of the ultrafiltrates and from actual shower waste was insignificant; waters S50, S53, S55A, L14, pooled Cincinnati tap water and distilled deionized water did not produce significant irritation under the circumstances of the test. The observed reactions to the two irritant samples showed a consistently different morphology and developed at different rates. Because of these differences, the relative degree of irritance assigned to the samples varied depending on the method used to score the overall irritation. No evidence of allergic contact sensitization was seen with either water; challenge patch testing was negative in both cases. Males appeared to be more easily irritated than females with both water samples. All testing was performed on the upper back; within this region of the skin relative patch position did not seem to be an important variable.

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# THE TOXICITY AND IRRITANCY OF ULTRAFILTRATES OF NON-SANITARY MILITARY WASTES

Final Report

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August 1, 1978

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# TOXICITY AND IRRITANCY OF ULTRAFILTRATES OF LAUNDRY AND SHOWER WASTE WATERS

SYLVAN O. WITHERUP, Edward A. Emmett, Department of Environmental Health, College of Medicine, University of Cincinnati, Cincinnati, Ohio.

## EXECUTIVE SUMMARY

A growing demand for water coupled with decreasing supply in many parts of the world has indicated a need to recycle and reuse water whenever possible. The continued reuse of limited water supply is feasible if hazards to health inherent to the accumulated wastes can be eliminated by efficient purification processes.

A process of membrane ultrafiltration and post chlorination has been developed to treat laundry and shower waste waters for potential recycle and reuse. Samples of water taken at various stages in the purification process have been evaluated in respect to their immediate toxicity when given orally to mice, as a primary skin irritant when kept in contact with the intact and abraded skin of rabbits and as an irritant to the ocular tissues in rabbits. Each of these tests provided quantitative data which correlated with the efficiency of the purification process.

The sample waters investigated were identified as: (1) synthetic laundry waste water, (2) synthetic or real shower waste water, (3) ultrafiltrates of Group 1, (4) ultrafiltrates of Group 2, and (5) concentrates (10 to 1000 fold) of waters from each of the 4 groups. The synthetic waste waters were constituted by adding to tap water known amounts of the chemical components identified in the real waste water, thereby providing for study water of known chemical composition which could be duplicated or altered as required. The various real wastes and ultrafiltrates were concentrated by a process of freezing.

Ninety-five water samples have been tested. The ultrafiltrates (including those concentrated by freezing) caused no irritation in the skin or eye of rabbits, and were not toxic when given orally to mice. Highly concentrated wastes

led to skin irritation ranging from mild erythema to necrosis and corrosion; evoked mild to severe conjunctivitis in the eye of rabbits and were toxic when given orally in massive dosage to mice. The severity of these responses was directly related to increasing concentration of waste chemicals as measured by the total organic carbon content of the water.

Ten of the water samples tested for mutagenic activity by the Ames test were all non-mutagenic; of these ten, six have been tested for irritation to the skin of volunteer human subjects.

Using a 21-day Continuous closed patch test technique in humans as a test for cumulative irritancy, two out of eight tested water samples namely S40 (100X synthetic shower waste) and L7 (50X synthetic laundry waste) were found to be significantly irritating. Irritation resulting from any of the ultrafiltrates and from actual shower waste was insignificant; waters S50, S53, S55A, L14, pooled Cincinnati tap water and distilled deionized water did not produce significant irritation under the circumstances of the test. The observed reactions to the two irritant samples showed a consistently different morphology and developed at different rates. Because of these differences, the relative degree of irritance assigned to the samples varied depending on the method used to score the overall irritation. No evidence of allergic contact sensitization was seen with either water; challenge patch testing was negative in both cases. Males appeared to be more easily irritated than females with both water samples. All testing was performed on the upper back; within this region of the skin relative patch position did not seem to be an important variable.

## FOREWORD

**In conducting the research described in this report, the investigators adhered to the principles described in the**

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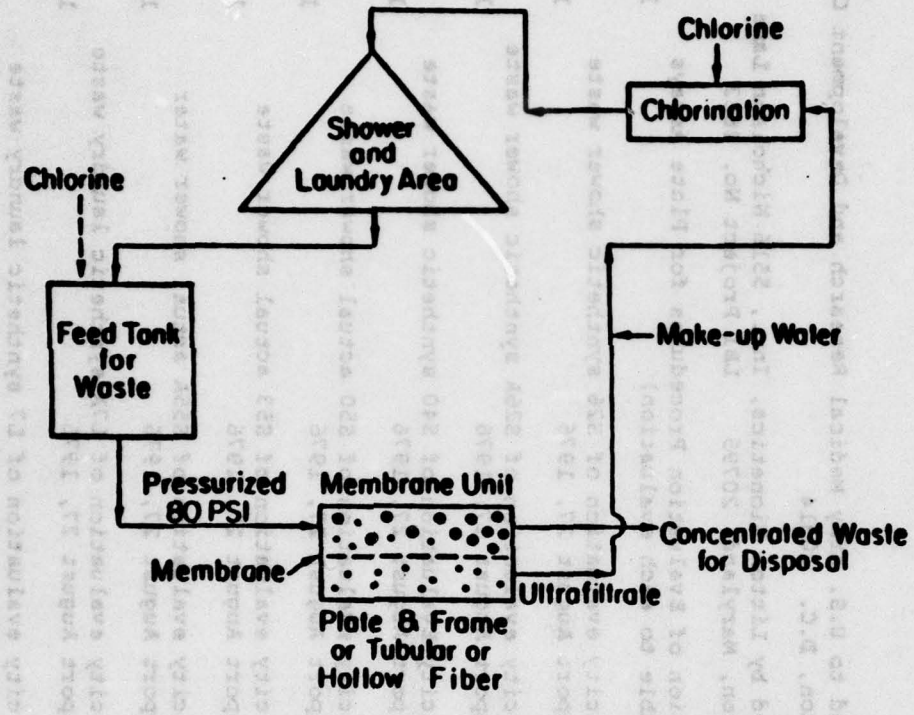


FIGURE 1. DIAGRAM OF A RECYCLE SYSTEM

## I. Introduction

A growing demand for water coupled with decreasing supply in many parts of the world has indicated a need to recycle and reuse water whenever possible. The continued reuse of limited water supply is feasible if hazards to health inherent to the accumulated wastes can be eliminated by efficient purification processes. The University of Kentucky in contract with the U.S. Army Medical Research and Development Command (DADA17-72-C-2050) has developed membrane ultrafiltration and post chlorination as a waste treatment and water renovation process for the potential recycle and reuse of laundry wastes, shower wastes and combined wastes containing both laundry and shower waste constituents.

In this research, toxicity tests served two purposes: First to detect changes in the toxic and irritant properties which were occasioned by changing the treatment process and which quantitated physiological effects in correlation with the efficiency of the treatment process. Secondly tests were needed also which would provide data suitable in part for judging the safety of water processed by the procedure.

## II. Materials

The technical details of the purification process have been described in the final report submitted by Dibakar Bhattacharyya and Robert B. Grieves (2), Department of Chemical Engineering, University of Kentucky. A schematic diagram of the system is presented in Figure 1 which may clarify the description of the water samples.

The sample waters investigated were identified as: (1) synthetic laundry waste water, (2) synthetic or real shower waste water, (3) ultrafiltrates of

**TABLE 1. The Composition of Synthetic IX (First Use) Shower and Laundry Wastes**

| Shower Waste | | Laundry Waste | |
|---|---|---|---|
| Component | mg/l | Component | mg/l |
| Soap (deodorant) | 70 | Nonionic surfactant (primary alcohol ethoxylates) | 100 |
| Shampoo | 40 | Carboxymethyl cellulose | 5 |
| Hair tonic | 25 | Oil | 200 |
| Tooth paste | 40 | $Ca^{(2+)}$ | 50 |
| Shower-lavatory cleaner | 100 | P | 100 |
| Disinfectant (sodium-o-phenyl phenolate) | 20 | Silicates | 100 |
| Insect repellant | 20 | Bleach ($Cl_2$) | 10 |
| Soil (0.2 clay) | 20 | Clay | 100 |
| Total organic carbon (TOC) content | | | |
| 86 mg/l | | 186 mg/l | |

Group 1, (4) ultrafiltrates of Group 2, and (5) concentrates (10 to 1000 fold) of waters from each of the 4 groups. The synthetic waste waters were constituted by adding to tap water known amounts of the chemical components identified in the real waste water, thereby providing for study water of known chemical composition which could be duplicated or altered as required. The various real wastes and ultrafiltrates were concentrated by a process of freezing.

The concentrations of the chemical components normally found in the respective wastes after one use are given in Table 1; these group concentrations expressed as 1X have been taken arbitrarily as a unit of waste resulting from a single use. Multiples of these 1X units represent the accumulated wastes resulting from repeated reuse of the water without any purification.

In all, ninety-five samples of water were tested. Shipped in lots of five or more from Lexington, Kentucky via United Parcel Service, they were received at the Kettering Laboratory the next morning. The samples were transferred from plastic containers to pyrex glass bottles, sealed and stored under refrigeration until used in the experiment. Usually five consecutively numbered samples were taken from the refrigerated supply, identified by number only and studied without reference to the components or treatment process.

The order in which the samples of water were submitted for study, their initial identification and the detailed test results obtained with each sample are contained in a series of progress reports submitted periodically to the U.S. Army Medical Research and Development Command, which have been included in in this report as Appendix B. A full description of each sample is provided in Table IA contained in Appendix A. A descriptive summary of all the samples is given in Table 2. In this tabulation, the first column shows the number of samples taken while processing a feed water containing the units of waste of Table I specified in the column headed Type of Sample; the remaining columns

TABLE 2. Description of Water Samples, their Number, Type and Source

| Number of Samples | Type of Sample | Total Organic Carbon ( TOC ) mg/l | | | |
|---|---|---|---|---|---|
| | | Waste Feed Water (F) | Ultrafiltrate (UF) | Concentrates Waste | Freeze |
| | | **Synthetic Shower Waste** | | | |
| 7 | 1X | 30-55 | 26 | 141 | 115-367(UF) |
| 1 | 5X | | | | 624(UF) |
| 10 | 10X | 204-210 | 49-113 | | 129-1052 |
| 3 | 20X | 1030 | | | |
| 4 | 30X | 1556-1620 | | | |
| 2 | 50X | 2181-3093 | | | |
| 4 | 100X | 2758-6044 | | | |
| 5 | 200X | 1556-4942 | | | |
| 2 | 300X | 15,000-18,000 | | | |
| 2 | 500X | 28,000 | | | |
| 2 | 1000X | 56,000 | | | |
| | | **Actual Shower Waste** | | | |
| 21 | 1X | 47-1215 | 19-68 | 104-1427 | 188-756(F) 147-337(UF) |
| | | **Synthetic Laundry Waste** | | | |
| | | **(A) With Neodol Detergent** | | | |
| 2 | 2X | | 4 | | 40(UF) |
| 1 | 5X | | 15 | | |
| 3 | 10X | 252 | 50 | | |
| 1 | 50X | 846 | | | |
| 1 | 75X | 930 | | | |
| 1 | 1X+20 g.(A)/l | 936 | | | |
| 2 | 100X | 2643 | | | |
| 3 | 500X | 72,000-106,250 | | | |
| 2 | 500X/2 | 42,000 | | | |
| 1 | 500X/3 | 24,000 | | | |
| | | **(B) With Army Type 1 Detergent** | | | |
| 1 | 1X + 20 g/l (B) | 986 | | | |
| 2 | 1X + 50 g/l | 1843 | | | |
| 1 | 1X + 100 g/l | 2687 | | | |
| 3 | 1X + 500g/l | 75,000-77,000 | | | |
| | | **Mixture of Synthetic Shower and Laundry Wastes (Equal Volumes 500X)** | | | |
| 1 | With Neodal (A) | 46,000 | | | |
| 1 | With AT1 detergent | 60,000 | | | |

**Special Samples**

| Number | Description | TOC (mg/l ) |
|---|---|---|
| 3 | Tap water, freeze concentrated | 23 - 110 |
| 2 | Na-O-phenyl phenolate (1000 mg/l) | 540 |
| 1 | Shower Cleanser (1000 mg/l) | 16 |

give the locations in the recycling system from which the samples were obtained and the extent to which some of the samples were concentrated by a freezing process; occasional wastes were diluted variously with tap water. The numbers under the various categories refer to the content (mg/l) of total organic carbon (TOC) found in the individual samples by chemical analysis at the University of Kentucky.

Male CF mice received in lots of 250 per week from Carworth Farms were distributed randomly, 5 per cage, among 50 cages contained on one rack. Male New Zealand white rabbits weighing 2½ to 3½ kg were obtained weekly in lots of 24 from various local rabbitrys; these were caged individually and were observed with respect to their activities for 4 or 5 days prior to their use.

## SECTION A - MAMMALIAN TESTING

### I. Methods

#### Oral Toxicity

After two weeks or more of observation with respect to their normal activity, 10 mice were weighed individually and given by intubation a dosage of 10, 25, 40, 63 or 100 ml/kg of a specific water; a total of 50 mice were given the respective dosages of any one water and 5 water samples were studied with each lot of mice. After dosage, the animals were observed daily during 14 days for signs of illness. All fatalities and any change in normal activity were noted in the records. The animals in each cage were weighed as a group on days 3, 7 and 14 after their dosage.

#### Primary Irritation

The ability of each water to produce primary irritation in the skin was measured according to the patch test technique described in paragraph I9I.I of Regulations under the Federal Hazardous Substances Act, Part I9I, Chapter I, Title 2I, Code of the Federal Regulations published by the U.S. Department of Health, Education, and Welfare, Food and Drug Administration (5) in I965 and as revised and ammended by the Consumer Product Safety Commission (4) in I973.

TABLE 3. Scoring Primary Skin Irritation

| Erythema and Eschar Formation | Score | Edema Formation | Score |
|---|---|---|---|
| No erythema | 0 | No edema | 0 |
| Very slight erythema (barely perceptible) | 1 | Very slight edema (barely perceptible) | 1 |
| Well defined erythema | 2 | Slight edema (edges of area well-defined by definite raising) | 2 |
| Moderate to severe erythema | 3 | Moderate edema (raised approximately one mm ) | 3 |
| Severe erythema (beet redness) to slight eschar formation (injuries in depth) | 4 | Severe edema (raised more than one mm and extending beyond the area of exposure) | 4 |

Summary Tabulation of Scores

| Condition | Time (hrs.) | Average score (*) | Condition | Time (hrs.) | Average score (*) |
|---|---|---|---|---|---|
| Erythema | | | Edema | | |
| Intact skin | 24 | ____ | Intact skin | 24 | ____ |
| " " | 72 | ____ | " " | 72 | ____ |
| Abraded skin | 24 | ____ | Abraded skin | 24 | ____ |
| " " | 72 | ____ | " " | 72 | ____ |
| Subtotal (S1) | | ____ | Subtotal (S2) | | ____ |

Primary irritation score = (S1 + S2)/4 = ____

(*) The score recorded in each of the eight categories is the average value for the six animals used in each test.

Test water (0.5 ml) was placed on a cotton swab about one square inch in area and placed in contact with the bare abdominal skin of a rabbit; two such patches were used with each water, one placed upon intact skin and the other upon an area of abraded skin on each of 6 rabbits. The patches were covered with a plastic sheet encircling the trunk of the animal and covered with a denim corset which kept the assembly in place and permitted the animal to be returned to its cage. After 24 hours, the coverings and patches were removed and the reactions in the skin were scored according to their severity as described in the procedural regulations summarized in Table 3.

Eye Irritation

The irritation produced in the eye following contact of the ocular tissues with a specific water was measured according to the definitive test described in paragraph 191.12 of the regulations cited above. Six rabbits were used for each test. Each eye was examined for redness and chemosis in the palpebral and bulbar conjunctivae and for any abnormality in the cornea or iris. If the ocular tissues were not normal in appearance the animal was excluded from the study. The test water was placed in one eye of each animal by gently pulling the lower lid away from the eye ball to form a cup into which 0.1 ml of the water was dropped. The lids were held together for one second and the animal released. The eyes were examined and the ocular reaction was recorded at 24, 48 and 72 hours. Grades for scoring the ocular lesions as defined in the Regulations are shown in Table 4.

## II. Results

Oral Toxicity - Mortality

Of the 95 samples submitted, 87 were administered orally to mice, the volumes of three synthetic shower wastes (S-63, 64, 65) and five laundry wastes (L-22, 23, 24, 25 and 26) being insufficient for the oral study. In the course of the experiments six mice were accidentally injured and were discarded; they were not included in the mortality data. Occasional mice escaped from their cages and were at liberty for a few or several hours; when recaptured they

TABLE 4. Grades for Scoring Ocular Lesions

| Rabbit Ident. Number | Graded Response Score | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 24 hours | | | 48 hours | | | 72 hours | | |
| | Conjunctiva. R * C | Cornea | Iris | Conjunctiva R * C | Cornea | Iris | conjunctiva R * C | Cornea | Iris |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |

**Conjunctiva**

* Redness
Refers to Palpebral and bulbar conjunctivae excluding cornea and iris.

Vessels normal---------- 0
Some vessels definitely injected---------- 1
Diffuse, crimson red, individual vessels not easily discernable--- (2)
Diffuse beefy red -------- 3

* Chemosis.

No swelling ---------- 0
Any swelling above normal (including nictitating membrane)----- 1
Obvious swelling with partial eversion of lids -(2)
Swelling with lids about half closed --------- 3
Swelling with lids more than half closed----- 4

**Cornea**

No ulceration or opacity-------- 0
Scattered or diffuse areas of opacity (other than slight dulling of normal luster)--- (1)
Easily discernable translucent areas, details of iris slightly obscured ---------- 2
Nacreous areas, no details of iris visible, size of pupil barely discernible --------- 3
Complete corneal opacity, iris not discernable ---------- 4

**Iris**

Normal---------- 0
Markedly deepened folds congestion, swelling, circumcorneal injection, sluggish reaction to light; any of these or any combination thereof ---------- (1)
No reaction to light, hemorrhage, gross distruction; any or all of these--------- 2

( ) Bracketed numbers indicate the lowest grades considered positive under the Federal Hazardous Substances Labeling Act Regulations.

were isolated for the remainder of the period of observation. All of the escapees lived and were included among the survival data; their weights were not included in the group body weight.

The fatalities resulting among mice after intubation of the respective wastes are summarized in Table 5 according to the source of the waste, the volume dosages administered and the range in their TOC contents. The mortality shown in the top four cells of Table 5 was in each instance apparently unrelated to the magnitude of the TOC dose administered and no peculiarity could be found in the components of the individual wastes which could account for the fatalities. Spontaneous deaths occurred also among undosed mice in the same lots from which the respective experimental groups had been constituted; this mortality ranged from 0.5 to 2.5 percent in specific lots, the incidence usually being as great or greater than in the intubated animals. This low sporadic mortality was attributed to extraneous diseases unrelated to ingestion of the respective wastes.

Four of the synthetic shower wastes (S-56, 57, 58, 59) and ten of the laundry wastes (consecutively numbered L-12 through 21) were found to be toxic. The TOC dose-mortality responses were evaluated by probit analysis of the combined data in the respective categories using the maximum likelihood method described by Finney (6). The TOC $LD_{50}$ values were 2382 and 2740 mg/kg for the synthetic shower and laundry wastes respectively; 100X or greater concentrations of either waste were required to achieve such dosages in mice.

Signs of Illness

Mice given a lethal dose of either waste became hypersensitive to auditory and tactile stimuli and exhibited increased ambulatory activity, mild tremors, occasional tonic convulsions, dyspnea and terminal coma. Mice given sublethal dosages exhibited less severe responses and also developed anorexia accompanied by decreased gain or actual loss in body weight.

No signs of illness were noted among mice given any of the ultrafiltrates

TABLE 5. The Mortality Among Mice According to the Volume Dose of Water and its Waste Contents

| Volume Dose ml/kg | Range in TOC Dose mg/kg | Fatilities * D T | Range in TOC Dose mg/kg | Fatalities * D T |
|---|---|---|---|---|
| | Tap water (3 samples) | | Real Shower Waste (21) | |
| 10 | 0.2-1.1 | 0-30 | 0.2-14. | 1-210 |
| 25 | 0.6-2.8 | 0-30 | 0.5-36. | 3-209 |
| 40 | 0.9-4.4 | 0-30 | 0.8-57. | 2-210 |
| 63 | 1.4-6.9 | 0-30 | 1.8-90. | 2-210 |
| 100 | 2.3-11. | 0-29 | 2.0-143. | 5-210 |
| Mortality | | 0-149 | | 13-1049 (1.2%) |
| | Low TOC Concentrations | | | |
| | Syn. Shower Waste (38) | | Laundry Waste (11) | |
| 10 | 0.2-60. | 5-378 | 0.04-18 | 0-110 |
| 25 | 0.4-151. | 1-379 | 0.1-46 | 0-110 |
| 40 | 0.6-242. | 1-378 | 0.2-74 | 2-110 |
| 63 | 1.0-381. | 2-379 | 0.3-116 | 0-110 |
| 100 | 1.6-604. | 5-381 | 0.4-184 | 4-110 |
| Mortality | | 14-1895 (0.7%) | | 6-550 (1.1%) |
| | Toxic TOC Concentrations | | | |
| | Syn. Shower Waste (4) | | Laundry Waste (10) | |
| 10 | 280-580 | 0-40 | 26-1065 | 0-100 |
| 25 | 700-1450 | 0-39 | 66-2662 | 17-97 |
| 40 | 1120-2320 | 7-39 | 106-4260 | 31-96 |
| 63 | 1764-3650 | 26-39 | 166-6710 | 41-95 |
| 100 | 2800-5800 | 30-37 | 264-10650 | 74-94 |
| LD50 | | 2382 mg/kg | | 2740 mg/kg |
| Fiducial limits | | {1664 mg/kg<br>{3410 mg/kg | | {1700 mg/kg<br>{4100 mg/kg |

TABLE 6. Wastes Irritating to the Eye of Rabbits.

| Sample number | Waste units | Total organic carbon | Positive Responses | Eye Irritant |
|---|---|---|---|---|
| **Lexington Tap Water Concentrated** | | | | |
| T8 | 0 | 110 | 3/12 | No |
| **Synthetic Shower Wastes** | | | | |
| S6 | 10X | 210 | 4/6 | Yes |
| S3 | 10X | 204 | 1/6 | No |
| S4 | 10X | 296 | 0/6 | No |
| S26 | 100X | 3602 | 4/6 | Yes |
| S59 | 500X | 28161 | 0/6 | No |
| S57 | (1000X)/2 | 28000 | 3/6 | Yes |
| S58 | 1000X | 56000 | 10/12 | Yes |
| **Synthetic Laundry Wastes** | | | | |
| L1, L4 | 10X | 252 | 4/18 | No |
| L12, L13 | 100X | 2643 | 8/12 | Yes |
| L15, L24 | 500X | 72000 | 12/12 | Yes |
| L18 | 1X +500 g/l ATI(*) | 75000 | 3/6 | Yes |
| **Equal Volume Mixtures of Shower & Laundry Wastes** | | | | |
| L17 = L15 + S59 | (500X + 500X)/2 | 46000 | 6/6 | Yes |
| L19 = L18 + S59 | | 60000 | 0/6 | No |

(*) ATI = Army Type 1 Detergent.

and their growing body weight was not affected by the waters ingested. Growth rate among the animals given the real shower waste was also normal.

Eye Irritation in Rabbits

In the progress reports (Appendix B) the eye irritation scores were reported as the total score elicited in each group of six rabbits and as the number of rabbits in each group of six or more which showed any reaction of sufficient severity to be classified as a positive response as defined in the regulations for judging eye irritation (cf. Table 4). The data showing the number of positive responses resulting from each type of waste, the original constitution of the feed water and the location from which the sample was taken in the purification process are summarized in Table 2A, Appendix A. Those wastes for which the positive responses were sufficient in number of classify the sample as an eye irritant are shown in Table 6.

No ultrafiltrate of any waste and none of the actual shower wastes including the feed water and the concentrates was found to be an eye irritant as defined by the regulations. An occasional individual response to these waters and to the less concentrated synthetic shower and laundry wastes consisted of moderate (Grade 2) erythema in the palpebral and bulbar conjunctivae with no swelling and no involvement of the cornea or the iris.

Contact of the ocular tissues with laundry wastes of 100X or greater concentration, synthetic shower waste of 1000X concentration and with a mixture containing equal volumes of 500X synthetic shower and laundry waste resulted in severe erythema and swelling with partial closure of the eyelids, opacity of the cornea (Grade 2) and occasional congestion and swelling in the folds of the iris. Recovery was complete in all instances within a period of seven days, the eye reacting normally to light stimulus and showing no corneal scars.

Primary Skin Irritation in Rabbits

The average skin response resulting in six rabbits after 24 hours of contact with each of the various actual and synthetic shower wastes is shown in Figure 2 in relation to the TOC content of the respective sample. The data pertaining to the laundry wastes are presented in like manner in Figure 3.

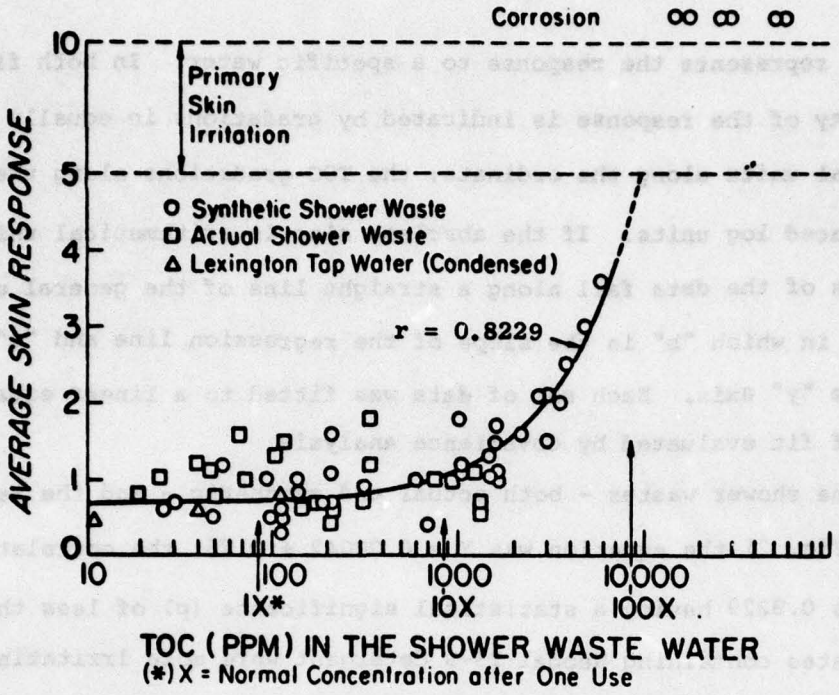


FIGURE 2. THE IRRITANCY OF SHOWER WASTE WATERS TO THE SKIN OF RABBITS

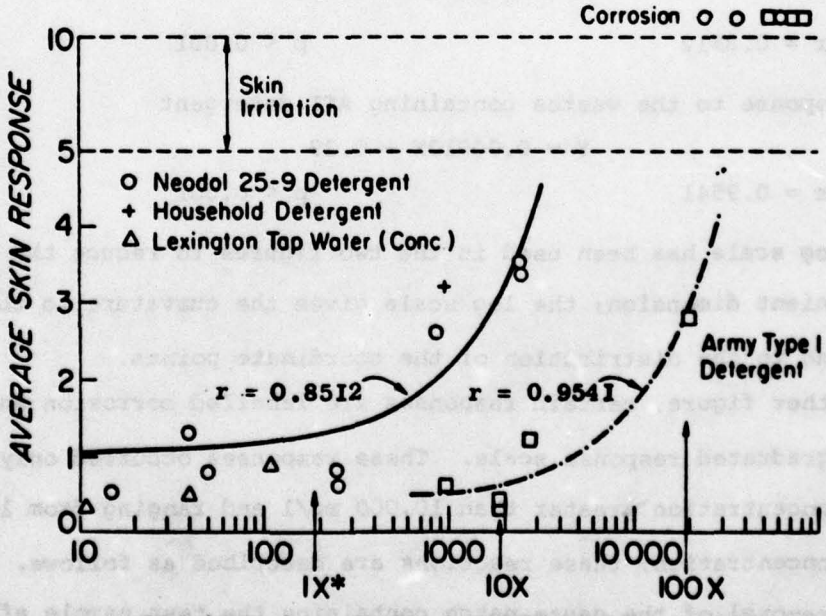


FIGURE 3. THE IRRITANCY OF LAUNDRY WASTE WATER TO THE SKIN OF RABBITS

Each point represents the response to a specific water. In both figures the severity of the response is indicated by gradations in equally spaced arithmetical units along the ordinate; the TOC gradations along the abscissa are equally spaced log units. If the abscissa also is arithmetical units, then the coordinates of the data fall along a straight line of the general equation $y = bx + c$ in which "b" is the slope of the regression line and "c" the intercept on the "y" axis. Each set of data was fitted to a linear equation and the goodness of fit evaluated by Covariance analysis.

For the shower wastes - both actual and synthetic - and the tap water controls (Fig. 2) the equation was $Y = 0.00042 + 0.75$; the correlation coefficient r was 0.8229 having a statistical significance (p) of less than 0.001. Laundry wastes containing Neodal 25-9 detergent were more irritating to the skin than those containing Army Type 1 (ATI) detergent; the differentiation was remarkable and the two sets of data were analyzed separately. For the response to the wastes containing neodal detergent:

$$Y = 0.00065X + 1.36$$

$r = 0.8512$ $\qquad$ $p < 0.001$

For the response to the wastes containing ATI detergent

$$Y = 0.00013X + 0.39$$

$r = 0.9541$ $\qquad$ $p < 0.001$

The log scale has been used in the two figures to reduce the abscissa to a convenient dimension; the log scale gives the curvature to the linear function and to the distribution of the coordinate points.

In either figure, certain responses are labelled corrosion and placed above the graduated response scale. These responses occurred only to samples with TOC concentration greater than 10,000 mg/l and ranging from 100X to 1000X in usage concentration; these reactions are described as follows.

Upon removal of the gauze patch containing the test sample after twenty-four hours of contact, the skin was found to be greyish white in color contrasting sharply with the normal pink skin; it was soft and pliable with but slight or no erythema nor edema in the surrounding tissue. Occasionally there were small non-hemorrhagic lesions in the exposed skin. After 72 hours

the skin was hard and dry occasionally fissured and with but slight or no reaction in the bordering tissues. This reaction was considered necrosis, and was arbitrarily labelled corrosion; there was no histological study of the tissues.

## IV. Mutagenicity Evaluation

Since its publication in 1971, the Ames test for detecting mutagenic chemicals (1) has reached a high level of popularity and has applied to a wide variety of chemicals in numerous laboratories. There is rapidly accumulating evidence that with few exceptions, carcinogens are mutagens. In a compilation of results obtained in various laboratories when the test was applied to 300 chemicals including known carcinogens and non-carcinogens, McCann, et al., (9) found that 90 percent of the known carcinogens were mutagenic. McCann and Ames (10) have reviewed the development of the test system and discussed recent improvements which have increased the sensitivity of the test.

In brief, compounds or suspect mixtures of compounds are tested on petri plates with specially constructed mutants of Salmonella typhimurium as the test strain. When a small amount of a mutagenic chemical is spotted on a lawn of the bacterial test strain in a petri dish, a positive result is seen by the growth of revertant bacteria around the spot.

For quantitative results different concentrations are tested individually by incorporating the mutagen into a thin agar overlay along with the bacteria; the numbers of revertent colonies are correlated with the corresponding concentration of the mutagen and dose-response curves obtained.

By adding a microsomal activation system of rat liver to the petri plates a variety of carcinogenic chemicals whose metabolites are the active agent are converted to mutagens and are easily detected.

Although the chemicals incorporated in the respective waste waters had long been used in commercial toiletries, soaps, deodorants, laundry detergents,

cleansers, etc., without apparent ill effect, still no information was found in the literature which demonstrated their harmlessness with continued usage. It seemed prudent, therefore, to test the waste waters for mutagenicity before applying them repeatedly upon the skin of human subjects.

Materials and Methods

After completion of the animal tests, ten waters that were non-toxic when given orally to mice, but which had elicited various degrees of irritation in the skin of rabbits were selected for mutagenicity testing. Of the ten waters, three were concentrated synthetic shower waste feed waters (SSWFC), one was actual shower waste concentrate (ASWC), two were ultrafiltrates (ASWF) of actual shower waste, three were synthetic laundry waste feed waters (SLWF) and one was a sample of Army type I (ATI) detergent. The identification of the waters and the results of the animal tests are given in Table 7. Certain duplicate or alternate preparations are noted in the table; for these the TOC values were somewhat higher than for the related water indicated in Table IA, Appendix A. Animal tests were not performed on two of the alternate samples but it was presumed that the results would have differed but slightly from those obtained with the original sample.

These samples were shipped by Dr. Bhattacharyya from the University of Kentucky, Lexington, Ky. to Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, Maryland 20795, who performed the tests.

Results of Tests

A complete report (7) on each of the ten waters, including the methods and materials employed, the test data, an interpretation of the data and the conclusions is supplied in Appendix C (page I50). It suffices to state here that each of the ten samples was found to be not mutagenic.

TABLE 7 - Identification and Description of Waters Tested for Mutagenicity

| Water Id. Number | Type of Waste | TOC PPM | Irritation in Rabbits Skin | Irritation in Rabbits Eye | Lethal Oral Dose ml/kg |
|---|---|---|---|---|---|
| S26 | SSWFC | 3602 | 2.0 | 4/6 | >100. |
| S26A | SSWFC | *3964 | | | |
| S40 | SSWFC | *8167 | 3.6 | 0/6 | >100. |
| S50 | ASWC | 1427 | 0.6 | 0/6 | >100. |
| S53 | ASUF | 51 | 0.8 | 0/6 | >100. |
| S55A | ASUF | * 200 | 1.6 | 0/6 | >100. |
| L7A | SLWF | * 456 | | | |
| L7 | SLWF | 800 | 2.6 | 0/6 | >100. |
| L12 | SLWF | 2643 | 3.5 | 3/6 | 100. |
| L14 | ATI | 2370 | 1.2 | 0/6 | >100. |

* A duplicate or alternate preparation; the TOC value is somewhat higher than in the original sample identified in Table IA, Appendix A.

## V. Discussion

The sporadic low incidence of mortality noted among the mice particularly in the early weeks of the experiments was apparently unrelated to the amount of waste in the dose administered and occurred at the same or higher rate among mice that had ingested none of the waste waters. The survival of large numbers of mice that had ingested much larger dosages of the same or more concentrated waste supports the opinion that the fatalities did not result from toxic effects of the respective wastes. Neither could they be attributed to a specific component of the wastes. Therefore, we have concluded that such fatalities resulted from extraneous disease.

Expression of the concentration of the waste components in terms of actual use provides some idea of the element of risk involved in partial or complete failure of the filtration membrane. The effects in animals, namely mortality, severe skin injury and eye irritation occurred only when the waste concentration

approached or exceeded 100 X. No adverse effect (as measured by these tests) resulted from any ultrafiltrate, any freeze concentrate of ultrafiltrate, or from any actual shower waste.

Likewise, as measured by a 21 day continuous patch test, ultrafiltrates and actual shower wastes were not irritating to the skin of human subjects. 50 X laundry waste and 100 X synthetic shower waste were severely irritating to human skin ( *see* section B, *pages* 37 *and* 40 )

It is noteworthy also that effects resulting from the two mixtures of shower and laundry wastes were in accord with those expected from the concentrations employed and provide no suggestion of synergism or potentiation.

All of the tests were concerned only with immediate injury resulting from the waste waters. No chronic or long-term feeding tests were performed. Such tests logically should be the next step in the program. The components of the respective wastes were materials that generally have been found safe for their respective purposes. Information regarding long-term effects of the products in low concentrations within a reuse system has not been found. It is significant, however, that ten waste waters in most instances concentrated samples of representative waters in the recycle system and ranging from mild to severe in their skin irritation score were in each instance found to be not mutagenic.

## VI. Conclusions

Actual shower waste water, ultrafiltrates of synthetic shower and laundry wastes and water containing the respective wastes in concentrations ranging up to five times the amounts expected from actual use (5 X) were not toxic when administered orally to mice in the largest dosage feasible, were not irritating to the eye of rabbits and elicited only minor and insignificant irritation in the skin of humans.

Irritation in the skin of rabbits was directly proportionate to the concentration of the total organic carbon (TOC) content of the respective wastes applied on the skin. The irritation was minor and of little physiological significance with TOC concentrations ten times greater than those resulting from actual use (I0 X). Severe skin injuries resulted from 50 and 100 X use

concentrations of either waste. Synthetic shower and laundry wastes in 10 X use concentrations were occasionally irritating to the eye of rabbits.

In 100 X concentrations, synthetic shower and laundry wastes were toxic when given orally to mice; in terms of TOC concentrations the respective $LD_{50}$ values were 2382 mg/kg and 2740 mg/kg. In 100 X concentrations the wastes also were severely irritating to the eye and skin of rabbits.

Three samples of synthetic shower waste concentrate, one sample of actual shower waste, two ultrafiltrates of actual shower waste and four samples of synthetic laundry shower waste taken as concentrated samples of representative waters in the recycle and ranging from mild to severe in their skin irritation score were in each instance found to be not mutagenic.

Using a 21-day Continuous closed patch test technique in humans as a test for cumulative irritancy, two out of eight tested water samples, namely S40 (100 X synthetic shower waste) and L7 (50 X laundry waste), were found to be significantly irritating. Waters S50, S53, S55A, L14, pooled Cincinnati tap water and distilled deionized water did not produce significant irritation under the circumstances of the test. The observed reactions to these two samples showed a consistently different morphology and developed at different rates. Because of these differences the relative degree of irritance assigned to these samples varied depending on the method used to score the overall irritation. No evidence of allergic contact sensitization was seen with either water, challenge patch testing was negative in both cases. Males appeared to be more easily irritated than females with both the water samples. All testing was performed on the upper back; within this region of the skin relative patch position did not seem to be an important variable.

## LIST OF TABLES AND FIGURES

## SECTION B - HUMAN SKIN IRRITATION TESTS

I. Introduction

After completion of the animal toxicity and irritation tests, ten water samples representing a range in the animal responses were tested for mutagenicity and each was found to be non-mutagenic (see page 24 and Appendix C, page I50). From among those ten, six waters were then jointly selected for human irritancy testing by Dr. Jack Dacre, U.S. Army Bioengineering Research and Development Laboratory, Dr. Dibaker Bhattacharyya, Department of Chemical Engineering, University of Kentucky and Dr. Edward Emmett, Department of Environmental Health. These waters were selected on the basis of their respective composition, lack of serious toxicity on animal testing and lack of mutagenicity by standard testing procedures.

II. Objective

To determine the relative potential of the waters to cause irritation in human skin by using a cumulative insult patch test technique.

III. Materials and Methods

A. Water Samples

Eight water samples were tested. Six samples provided by the University of Kentucky were shipped from Lexington, Kentucky via United Parcel Service upon receipt. The samples were transferred from plastic containers to pyrex glass bottles, sealed and stored in a refrigerator until used in the experiments. In order to minimize handling and changes in temperature, aliquots for several days use were transferred after careful mixing to smaller bottles for day by day use. These bottles were also refrigerated between use. Each sample was warmed to redissolve any precipitate and was thoroughly mixed before any aliquot was removed. Samples were identified by number only and studied without reference to the components or treatment process. These waters are listed and described as waters 1 to 6 in Table 8. The sample number is the number given to identify

TABLE 8 - List and Description of Water Samples Used for Human Cumulative Insult Patch Testing

| Water No. | Sample Identity Number | Description of Sample | TOC | (a) TS |
|---|---|---|---|---|
| 1 | S40 | Concentrated synthetic shower waste feed (unfiltered) | 8167 | 20,800 |
| 2 | S50 | Concentrated actual shower waste feed | 1427 | 4,020 |
| 3 | S53 | Actual shower waste ultrafiltrate | 51 | 280 |
| 4 | S55A | Actual shower waste ultrafiltrate | 200 | 727 |
| 5 | L7 | Synthetic laundry waste feed concentrated | 800 | 28,000 |
| 6 | L14 | Concentrated U.S. Army detergent | 2370<br>23 | 55,000 |
| 7 | None | Deionized distilled water | | |
| 8 | None | Pooled shower water | | |

(a). Total Organic Carbon (TOC) and Total Solute (TS) Levels (PPM)

TABLE 9 - Summary of Results of Animal Tests

| Water No. | Water Identity Number | $LD_{50}$ Mice | Rabbit Eye Irritation Score (b) | Rabbit Skin Irritation Avg. Score | Mutagenicity |
|---|---|---|---|---|---|
| 1 | S40 | >100 ml/kg | 6 | 3.6 | Negative |
| 2 | S50 | >100 ml/kg | 5 | 0.6 | Negative |
| 3 | S53 | >100 ml/kg | 8 | 0.83 | Negative |
| 4 | S55A | >100 ml/kg | 11 | 1.63 | Negative |
| 5 | L7 | >100 ml/kg | 10 | 2.6 | Negative |
| 6 | L14 | >100 ml/kg | 7 | 1.23 | Negative |

(b) Total score among 6 rabbits. Very slight or minor irritation; not sufficient in any instance to qualify as a positive response.

them in other parts of the contract. The results of the toxicological testing of these water samples in animals are summarized in Table 9.

A 7th water sample consisted of deionized distilled water as usually prepared in the Department of Environmental Health, University of Cincinnati. It was stored and handled in the same manner.

The 8th sample was of pooled Cincinnati tap water. This was collected from a shower at the Cincinnati General Hospital, at the heat usually used for showering. The water was let run for 2 minutes, then 150 ml. was collected. Such collections were made every day for 5 days. The collections were pooled and stored and handled in the same manner as the other samples.

B. Subjects

Twenty-one volunteers took part in the cumulative insult patch test program. Ten subjects were females, two Negro and eight Caucasian, with a mean age of thirty-one. Eleven subjects were male, two Negro and nine Caucasian with a mean age of thirty. The demographic characteristics of the subjects are shown in Table 10. The informed consent form agreed to by the volunteers is shown in Table IIIA, Appendix A.

A directed medical history was taken from each volunteer and an examination of the skin was performed. Subjects with active skin disease, a history of severe dermatitis or currently under medical treatment were excluded from testing.

C. Testing Methods

(1) 21-Day Continuous Closed Patch Test

The method used was a modification of the 21 day continuous closed patch test (8,II,I2). The subjects were studied in two groups, a first group of ten (five males and five females) and a second group of eleven (six males and five females). Two by two cm patches of nonwoven fabric ( Webril®),were impregnated with 0.2 ml of the test fluid dispensed by a glass tuberculin syringe. Care was taken to ensure that the water samples from which aliquots were taken were evenly dispersed. Patches of each of the eight water samples were placed

**TABLE 10 - Demographic Characteristics of Subjects**

| Group 1 Subjects | | | | Group 2 Subjects | | | |
|---|---|---|---|---|---|---|---|
| Identify Symbol | Sex | Race | Age | Identify Symbol | Sex | Race | Age |
| A | F | C | 24 | K | F | C | 51 |
| B | F | N | 30 | L | F | C | 26 |
| C | F | C | 32 | M | F | C | 28 |
| D | F | C | 30 | N | F | C | 24 |
| E | F | C | 33 | O | F | N | 32 |
| F | M | C | 27 | P | M | N | 49 |
| G | M | N | 33 | Q | M | C | 31 |
| H | M | C | 26 | R | M | C | 25 |
| I | M | C | 31 | S | M | C | 25 |
| J | M | C | 28 | T | M | C | 28 |
| | | | | U | M | C | 30 |

**TABLE 11 - Scoring Criteria for Observed Reactions**

0 - No reaction

± - Questionable; ill-defined erythema not covering entire patch area

1 - Erythema with a definite margin in patch area

2 - Erythema with induration or cracking

3 - Vesiculation, pustule formation or fissuring

4 - Application of the test water was discontinued when the subject reached a Grade 3 reaction, and this score was used for each of the days remaining in the 21 day period

on paraspinal locations on each subject's upper or midback, four on each side of the midline. The patches were occluded with impermeable plastic tape (Blenderm®, Minnesota Mining and Manufacturing Corporation). At the end of 24 hours the patches were removed, the test site was read 30 minutes later, and then freshly prepared patches were reapplied to the same area. Patches were applied daily for 21 days.

Reactions were scored as shown in Table 11. Other types of reactions such as scaling or changes in pigmentation and subjective responses such as itching or burning were also recorded. An example of the form used for recording reactions is shown in Table IVA, Appendix A. Testing at a given site was continued until a Grade 3 reaction was obtained.

In the first group of subjects the relative positions of the various tested waters on the back remained constant in all individuals; in the second group of subjects the order was changed.

Subjects in the study were instructed to avoid prolonged immersion of the patched area while the patches were *in situ* but had no other restrictions.

(2) Challenge Patch Testing

Challenge patch testing was performed with samples 1 and 5 on 6 subjects in the second tested group, in order to determine if the reactions observed to these waters were due to allergic sensitization. Patches impregnated with each water could be applied to a previously untested area of the skin of the upper back in the same manner as described above. The patches were removed 48 hours later and were observed and read 30 minutes later and again 72 hours after application.

D. Computation of Irritancy

Two different approaches were used to compute the irritancy of the individual samples in order that the results could be compared.

(1) Cumulative Irritation Index

This index was derived in the manner described by Phillips *et al.* (11).

Daily scores for each material are added and the sum of the daily scores for each subject divided by the total number of subjects. The ± and 0 scores are assigned no numerical value. As testing was discontinued when a score of 3 was reached this score was assigned to each subsequent reading for that material. The maximum cumulative irritation index which could be obtained for this test in our study was 63, and the least was 0.

(2) Cumulative Percent Reacting

The cumulative percentage of subjects reacting each day can be represented graphically (3). We used each of three end points namely a 1, 2 and 3 scored reaction in plotting such a relationship.

(3) 50% Irritation Time

From a graph of the cumulative percentage of subjects reacting each day, the time taken for 50% of subjects to develop significant irritation can be computed. More precisely the time taken for 50% of subjects to reach each reaction score; i.e., a 1, 2 or 3 score reaction can be computed. We have designated these points as:

Irritation time for 1 score reaction in 50% of subjects ($I_1T_{50}$)

Irritation time for 2 score reaction in 50% of subjects ($I_2T_{50}$)

Irritation time for 3 score reaction in 50% of subjects ($I_3T_{50}$)

In order to distinguish such values determined for humans from those determined for experimental animals the postscript human could be added. Thus a complete abbreviation could read $I_1T_{50}$ (human).

IV. Approval by Faculty Committee on Human Research

Approval of this study was obtained from the Faculty Committee on Human Research at the University of Cincinnati.

## V. Results

### A. Study Progress

Experimental subject compliance with the protocol was good. During the first several days of the experiment most subjects reported mild to moderate pruritus over the entire area in contact with the Blenderm® tape. With most subjects this improved, but one male subject withdrew from the study after 4 days because of severe pruritus experienced after riding a certain distance on his bicycle each day. The results of the incomplete testing on this subject are not reported further here. A female subject developed an erythematous reaction to the tape which was very irritating and she withdrew from the study after the 16th application.

With the exception of one water sample, patches adhered well. In general patches placed higher on the back adhered slightly better. Sample No. 6, the concentrated U.S. Army detergent slipped from its original position on an average of 8 out of the 21 applications on each subject. The patch generally remained within 1 cm of the original application site and in no case contaminated the site of application of another patch. The slipping of patches impregnated with this sample was independent of the position on the back and appeared to be due to the nature of the water sample.

### B. Relative Irritancy of Waters

#### 1) Results with Individual Waters

**Sample 1**

This sample, concentrated synthetic shower waste, produced significant irritation on all subjects. It produced a characteristic response which initially consisted of erythema which progressed to deeper erythema, induration,

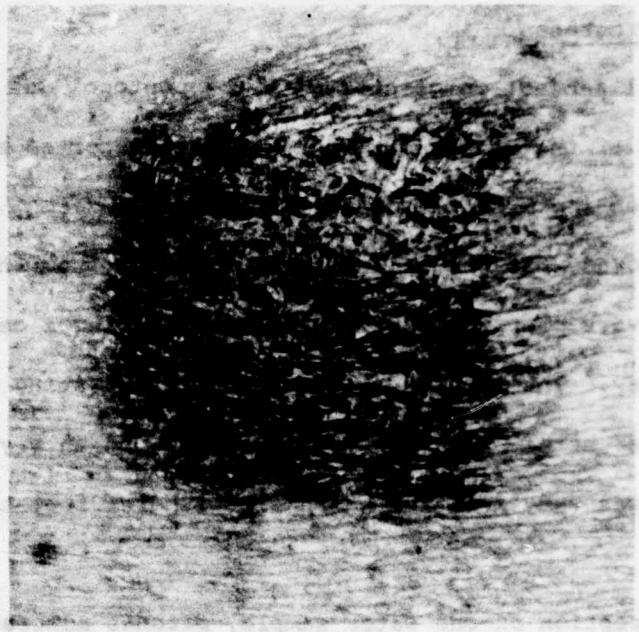

**Figure 4. Irritation to Human Skin Resulting from Sample 1, (S-40) 100X Synthetic Shower Waste.**

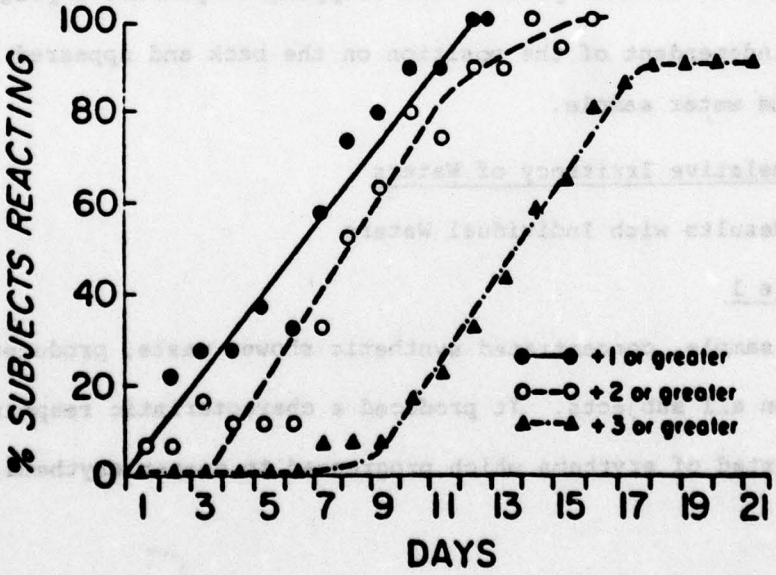


**FIGURE 5. CUMULATIVE PERCENTAGE OF SUBJECTS WITH GRADE 1, GRADE 2, OR GRADE 3 REACTION TO SAMPLE 1 (S-40).**

scaling, cracking then painful fissuring. No vesicles or pustules were observed as a reaction to this sample. The resolution of these changes generally took a week or more after application of this sample ceased. In several subjects hyperpigmentation was noted over the area tested with this water as the reaction resolved. The appearance of a typical reaction to sample 1 is shown in Figure 4.

The sequential pattern of response to water sample 1 was as follows: A grade 1 or greater reaction developed in half the subjects by day 6 and in all subjects by day 12. The average time of progression from a grade 1 reaction to a grade 3 reaction was 7 days. The percentage of subjects exhibiting a grade 1 response or greater, a grade 2 response or greater and a grade 3 response to sample 1 on each day of the study is shown in Figure 5.

Using the data from the 10 males and 9 females who completed the study, the Cumulative Irritation Index for S40 was 31.6 in females, 40.3 in males and 36.2 for all subjects combined. For all subjects combined the $I_1T_{50}$ was 6 days, the $I_2T_{50}$ was 8 days and the $I_3T_{50}$ 13.5 days.

Sample 2

This sample, concentrated actual shower waste water, produced a single Grade 1 response in each of 4 subjects and a single grade 2 response in one female subject. The Cumulative Irritation Index for Sample 2 was 0.5 for females, 0.1 for males, and 0.3 for both sexes combined. The $I_1T_{50}$ was greater than 21 days.

Sample 3

This sample, actual shower waste ultrafiltrate, produced a single Grade 1 reaction in each of 4 female subjects and no significant reactions in the male subjects. The Cumulative Irritation Index for Sample 3 was 0.4 for females, 0 for males, and 0.2 for both sexes combined. The $I_1T_{50}$ was greater than 21 days.

Sample 4

This sample, actual shower waste ultrafiltrate, caused a single Grade 1 reaction in each of 3 female subjects, three grade 1 reactions in one male subject and 4 days of a Grade 1 reaction in another. These reactions were transient, did not progress, and reverted to negative readings. The Cumulative Irritation Index for sample 4 was 0.3 for females, 0.8 for males, and 0.5 for both sexes combined. The $I_1T_{50}$ was greater than 21 days.

Sample 5

This sample, synthetic laundry waste feed concentrate, produced a characteristic response which began with perifollicular inflammation followed by the development of pustules. In some cases pustules developed without previous erythema or papules being observed. The uniform induration, cracking or fissuring seen as a reaction to Sample 1 was not produced in response to the application of Sample 5. The appearance of a typical reaction to sample 5 is shown in Figure 6. An average of 10 days of application was required to produce a Grade 1 or greater response in half the subjects. After 16 days application of Sample 5 all subjects exhibited a Grade 1 or greater reaction. The average time necessary for subjects to progress from a Grade 1 to Grade 3 response was only 2 days, reflecting the rapid development of pustules. The Cumulative Irritation Index for Sample 5 was 25.8 for females, 35.1 for males and 30.7 for both sexes combined. The cumulative percentage of subjects exhibiting a Grade 1 or greater, a Grade 2 or greater, and a Grade 3 response to Sample 5 on each day of reading is shown in Figure 7. The $I_1T_{50}$ was 10 days, $I_2T_{50}$ was 11 days and $I_3T_{50}$ 11.5 days.

Sample 6

This sample, concentrated U.S. Army detergent, produced a Grade 2 reaction

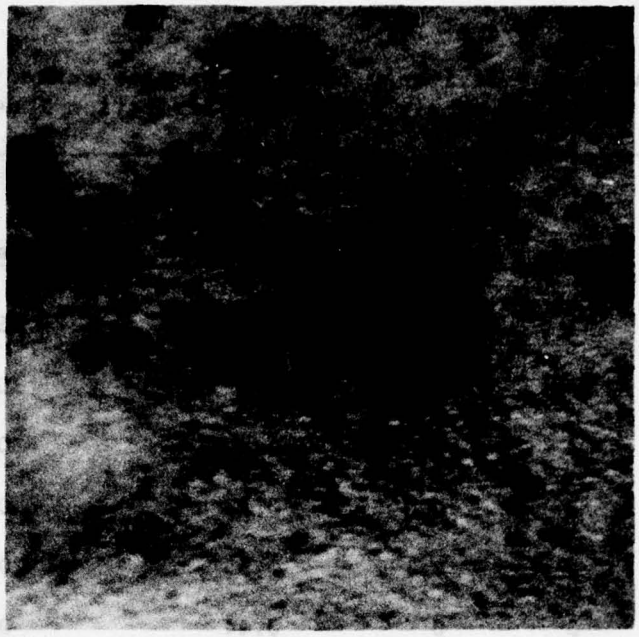

Figure 6. Irritation to Human Skin Resulting from Sample 5, (L-7) 50X Laundry Waste.

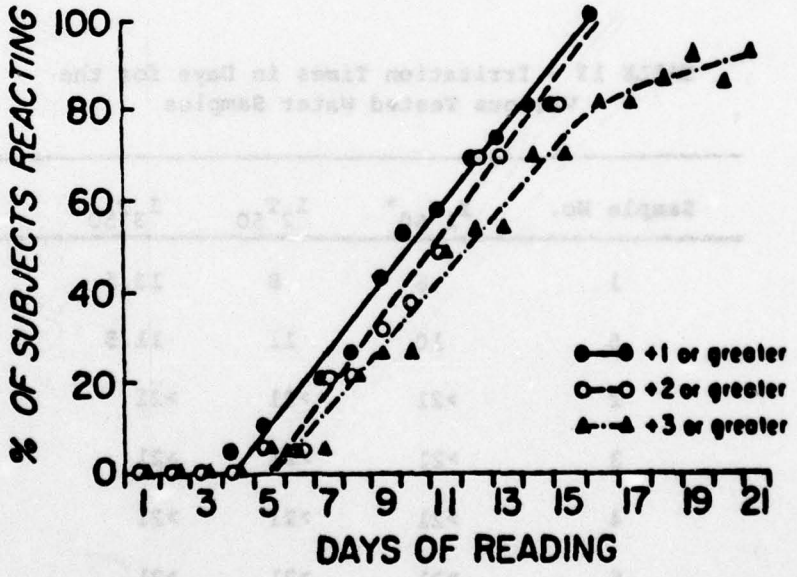


FIGURE 7. CUMULATIVE PERCENTAGE OF SUBJECTS WITH GRADE 1, GRADE 2, OR GRADE 3 REACTION TO SAMPLE 5 (L-7).

TABLE 12 - Irritancy of Water Samples Ranked According to Their Cumulative Irritation Index

| Sample | Cumulative Irritation Index | | |
|---|---|---|---|
| | Males | Females | Both Sexes Combined |
| 1 | 40.3 | 31.6 | 36.2 |
| 5 | 35.1 | 25.8 | 30.7 |
| 4 | 0.8 | 0.33 | 0.58 |
| 6 | 0.3 | 0.44 | 0.37 |
| 2 | 0.1 | 0.55 | 0.32 |
| 3 | 0 | 0.44 | 0.21 |
| 7 | 0.1 | 0 | 0.05 |
| 8 | 0 | 0 | 0 |

TABLE 13 - Irritation Times in Days for the Various Tested Water Samples

| Sample No. | $I_1T_{50}$* | $I_2T_{50}$ | $I_3T_{50}$ |
|---|---|---|---|
| 1 | 6 | 8 | 13.5 |
| 5 | 10 | 11 | 11.5 |
| 2 | >21 | >21 | >21 |
| 3 | >21 | >21 | >21 |
| 4 | >21 | >21 | >21 |
| 6 | >21 | >21 | >21 |
| 7 | >21 | >21 | >21 |
| 8 | >21 | >21 | >21 |

*See text for definition of symbols

in one female on the final 2 days of the study and a Grade 1 reaction on days 19 and 20 in one male subject. All other readings were negative. The Cumulative Irritation Index for Sample 6 was 0.4 for females, 0.3 for males, and 0.3 for both sexes combined. The $I_1T_{50}$ was greater than 21 days. As noted patches impregnated with this sample adhered poorly to the Blenderm® tape and showed a marked tendency to slip. The extent to which this may have influenced the observed results is unknown.

Sample 7

This sample, deionized water, produced no reactions except for one Grade 1 reaction in one male subject for one day. The Cumulative Irritation Index for this sample was 0 for females, 0.1 for males and 0.05 for both sexes combined. The $I_1T_{50}$ was greater than 21 days.

Sample 8

This sample, pooled shower water, produced no reactions in any of the subjects. Consequently its Cumulative Irritation Index for males, females and both sexes combined was 0. The $I_1T_{50}$ was greater than 21 days.

(2) Comparison of Irritancy of Individual Waters

The irritancy of the various waters ranked according to their cumulative irritation index is shown in Table 12. It will be seen that Sample 1 was the most irritating, Sample 5 was somewhat less irritating, but whereas these samples both produced a significant degree of irritation the other 6 samples produced very low order or no irritation.

A comparison of the irritation times for 50% of subjects for the different samples is shown in Table 13. It will be seen that only to waters 1 and 5 could be attributed finite irritation times. If responses with a score of 1 or 2 were used as the basis for comparison, water I was significantly more irritating

**TABLE 14 - Results of Responses to the Initial Application and of Challenge Testing to detect Possible Allergic Sensitization**

| | Sample 1 | | | Sample 5 | | |
|---|---|---|---|---|---|---|
| | Initial Application | Challenge Application | | Initial Application | Challenge Application | |
| Subject | 48 hr. | 48 hr. | 72 hr. | 48 hr. | 48 hr. | 72 hr. |
| N | 0 | 0 | 0 | 0 | 0 | 0 |
| M | 0 | ± | 0 | 0 | 0 | 0 |
| K | 2 | 0 | 0 | 0 | 0 | 0 |
| T | 0 | 0 | 0 | 0 | 0 | 0 |
| P | 1 | 1 | 0 | 0 | 0 | 0 |
| Q | 1 | ± | 0 | 0 | ± | 0 |

**TABLE 15 - Cumulative Irritation Indices for the 4 Positions on the Back**

| Position | Cumulative Irritation Index | | |
|---|---|---|---|
| | Water 1 | Water 5 | Average both waters |
| 1 (uppermost) | 35.2 | 35.2 | 35.2 |
| 2 | 32.3 | 28.0 | 30.2 |
| 3 | 42.3 | 29.3 | 35.8 |
| 4 (lowest) | 36.7 | 23.3 | 30.0 |

than water 5, whereas water 5 was the most irritating if the time taken to reach a score of 3 in 50% of individuals was used as the basis for comparison. These results reflect the difficulties inherent in comparing morphologically different types of responses.

C. Challenge Testing for Allergic Sensitization

In order to exclude the possibility that the observed reactions were due to allergic sensitization, 6 subjects where challenge tested with samples 1 and 5 applied for 48 hours as described in the methods section. The results of testing are shown in Table 14. No significant alterations in reactivity to samples 1 and 5 were observed with challenge testing in any of the subjects tested.

D. Effect of Sex

It will be seen in Table 12 that males had a higher cumulative irritation index than females for both Sample 1 and Sample 5, the difference being statistically significant ($p < 0.01$) in each instance.

E. Effect of Sites

To determine the effects of the position of a sample on the back, the sites of application were rotated in the second group of 10 subjects. Table 15 shows a comparison of the Cumulative Irritation Indices for the 4 positions on the back. Although there was some variation in the results no consistent trend was seen to suggest that position on the back was an important variable.

## VI. Conclusions

Using a 21-day Continuous closed patch test technique in humans as a test for cumulative irritancy, two out of eight tested water samples, namely S40 and L7, were found to be significantly irritating. Waters S50, S53, S55A, L14, pooled Cincinnati tap water and distilled deionized water did not produce significant irritation under the circumstances of the test. The observed reactions

to these two samples showed a consistently different morphology and developed at different rates. Because of these differences the relative degree of irritance assigned to these samples varied depending on the method used to score the overall irritation. No evidence of allergic contact sensitization was seen with either water; challenge patch testing was negative in both cases. Males appeared to be more easily irritated than females with both the water samples. All testing was performed on the upper back, within this region of the skin relative patch position did not seem to be an important variable.

## LIST OF TABLES AND FIGURES

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## APPENDIX A



### Explanatory Notes

**Table IA:**

"S" in the sample number denotes shower waste, either actual or synthetic.

"L" in the sample number denotes laundry waste or some specific component of laundry waste.

LI7 and LI9 samples were equal volume mixtures of shower and laundry waste.

SI and S7 samples were unchlorinated; all other samples were properly chlorinated unless otherwise specified.

Among some of the samples concentrated by freezing, the actual Total Organic Carbon (TOC) contents did not increase proportionately with the decrease in water volume; note the TOC values for SI and S2. TS indicates Total Solutes.

A dilution factor of a:I means a factor of a + I.

All of the water samples were within the pH range of 7 to 8.

**Table IIA:**

In this tabulation, the respective wastes are listed in terms of the initial usage units (Type of Water), number of samples (N), the location in the recycle system from which the samples were taken, their TOC content and whether freeze concentrated or not.

Under the heading R(+) in the tabulation is given the number of rabbits showing significant eye irritation ( positive response) among the total number tested with the specific water; those which were sufficient to classify the waste as an eye irritant are marked with an asterisk (*).

TABLE IA: Description of Water Samples

| Sample Number | Type of Sample | TOC mg/l | TS mg/l |
|---|---|---|---|
| S1 | Synthetic 1X, filtered (0.45 μ) | 55 | 260 |
| S2 | Synthetic 1X, filtered, then freeze* concentrated 10:1 (by volume) | 367 | 2200 |
| S3 | Synthetic 10X, filtered | 204 | 460 |
| S4 | Synthetic 10X, filtered, then freeze concentrated 1.30:1 | 296 | 440 |
| S5 | Synthetic 10X, ultrafiltered (PSAL) | 98 | 250 |
| S6 | Synthetic 10X (without Ajax, disinfectant, DEET), filtered | 210 | 340 |
| S7 | Ultrafiltrate (PSAL) of unfiltered S6 | 49 | 130 |
| S8 | Chlorinated S5 | 98 | 270 |
| S9 | Actual waste, filtered | 47 | - |
| S10 | S9, freeze concentrated 12:1 | 188 | - |
| S11 | Ultrafiltrate of actual waste | 25 | 150 |
| S12 | S11 freeze concentrated 10:1 | 147 | 2125 |
| S13 | Actual waste, ultrafiltrate (PSAL) | 19 | 210 |
| S14 | Synthetic 10X, ultrafiltrate (PSAL), unchlorinated | 113 | - |
| S15 | Chlorinated S14 | 113 | - |
| S16 | Synthetic 10X, filtered, then freeze concentrated 10:1 | 1052 | 3200 |
| S17 | Synthetic 1X, ultrafiltrate (Fluxo) | 26 | 100 |
| S18 | Synthetic 20X, filtered | 1030 | 1580 |
| S19 | Synthetic 50X, filtered, unchlorinated | 3093 | 4500 |
| S19A | Synthetic 50X, filtered | 3093 | - |

TABLE IA: ( Continued)

| Sample Number | Type of Sample | TOC mg/l | TS mg/l |
|---|---|---|---|
| S20 | Actual waste, filtered, freeze concentrated 20:1 | 340 | 4850 |
| S21 | Actual waste, ultrafiltrate (PSAL), then freeze concentrated 12:1 | 337 | 4050 |
| S22 | Synthetic 5X, ultrafiltrate (PSAL) at high water recovery, then freeze concentrated 20:1 | 624 | 4200 |
| S23 | Actual waste, filtered, freeze concentrated 20:1 | 215 | 3817 |
| S24 | Synthetic 20X, ultrafiltrate (PSAL), freeze concentrated 15:1 | 704 | 1970 |
| S25 | Actual waste, ultrafiltered (PSAL), freeze concentrated 38:1 | 202 | 4712 |
| S26 | Synthetic 100X, filtered | 3602 | 10,396 |
| S27 | 1000 mg/l Na-ortho-phenyl-phenolate | 540 | - |
| S28 | S27 + 100 mg/l $Cl_2$ | 540 | - |
| S29 | 1000 mg/l Ajax, filtered | 16 | 228 |
| S30 | Synthetic 30X, filtered, unchlorinated | 1620 | 4792 |
| S31 | Synthetic 30X, filtered | 1620 | 5326 |
| S32 | Same as S31 | 1620 | 5326 |
| S33 | Same as S31 | 1620 | 5326 |
| S34 | Synthetic 1X, concentrate (UOP225) at 73% water recovery, filtered | 141 | 355 |
| S35 | Synthetic 1X, ultrafiltrate (UOP225) at 73% water recovery, then freeze concentrated 42:1 | 115 | 6065 |
| S36 | Same as S34 | 141 | 355 |
| S37 | Synthetic 100X (without phenyl-phenolate), filtered | 3748 | - |
| S38 | Synthetic 1X, filtered | 30 | 330 |
| S39 | Synthetic 10X, filtered, then freeze concentrated 9:1 | 602 | 2480 |
| S40 | Synthetic 100X, unfiltered | 6044 | 27,388 |
| S41 | Exact duplicate of S18 | 1030 | 1580 |

Table IA: (Continued)

| Sample Number | Type of Sample | TOC mg/l | TS mg/l |
|---|---|---|---|
| S42 | Actual waste, ultrafiltrate (UOP225), then concentrated by evaporation at temp. 30-35°C, 35:1 by volume | 309 | 8512 |
| S43 | Synthetic 200X, filtered | 4942 | 9500 |
| S44 | Synthetic 200X, diluted 1:1, filtered | 2738 | 4418 |
| S45 | Synthetic 200X, diluted 1.5:1, filtered | 2181 | - |
| S46 | Synthetic 200X, diluted 3.0:1, filtered | 1556 | 2174 |
| S47 | Same as S46 | - | - |
| S48 | Actual waste, concentrate (PSAL cassette) at 85% recovery, filtered | 104 | 793 |
| S49 | Actual waste, ultrafiltrate (UOP225) at high recovery | 40 | 307 |
| S50 | Actual waste, concentrate (UOP225) at 93% recovery | 1427 | 4020 |
| S51 | Actual waste with urine, filtered, then freeze concentrated 20:1 | 746 | - |
| S52 | Actual waste with urine, filtered | 72 | 464 |
| S53 | Actual waste with urine ultrafiltrate (PSAL) | 51 | 280 |
| S54 | Actual waste, concentrate (3rd pass, PSAL cassette) at 92% recovery, filtered | 116 | - |
| S55 | Actual waste, ultrafiltrate (3rd pass, PSAL cassette) at 92% recovery | 68 | 500 |
| S56 | Synthetic 1000X, unfiltered | 58,000 | 96,000 |
| S57 | S56 diluted 1:1 | 28,000 | 47,000 |
| S58 | Synthetic 1000X, filtered | 56,000 | 71,500 |
| S59 | Synthetic 500X, filtered | 28,161 | 41,630 |
| S60 | Actual waste, concentrate (7th pass DP06 membrane) at 90% recovery | 941 | 3865 |
| S61 | High concentration, actual waste, unfiltered | 1215 | 1156 |
| S62 | S61 filtered | - | 564 |

TABLE IA: (Continued)

| Sample Number | Type of Sample | TOC mg/l | TS mg/l |
|---|---|---|---|
| S63 | S56 diluted 3:1 | 15,000 | - |
| S64 | S57 diluted 3:1 | - | - |
| S65 | S58 diluted 3:1 | 18,000 | - |
| T8 | Lexington tap water freeze concentrated 33:1 | 110 | 3130 |
| T9 | Lexington tap water freeze concentrated 20:1 | 40 | - |
| T10 | Lexington tap water freeze concentrated | 23 | 3830 |
| L1 | Synthetic 10X, filtered | 252 | 7550 |
| L2 | Synthetic 10X, ultrafiltrate (Fluxo) | 50 | 4830 |
| L3 | Synthetic 5X, ultrafiltrate (PSAL) at high recovery | 15 | 1013 |
| L4 | Same as L1 | | |
| L5 | Synthetic 2X, ultrafiltrate (PSAL) at high recovery | 4.0 | 510 |
| L6 | L5 freeze concentrated 10:1 | 40.0 | - |
| L7 | Synthetic 50X, filtered | 846 | 28,000 |
| L8 | Synthetic waste with 20.0 gm/l Army Type I detergent, filtered | 986 | 2,476 |
| L9 | Same as L8 but using TIDE detergent | 936 | 16,322 |
| L10 | Synthetic waste with 50.0 gm/l Army Type I detergent, filtered | 1843 | 19,500 |
| L11 | Same as L10 | - | - |
| L12 | Synthetic 100X, filtered | 2643 | 72,340 |
| L13 | Same as L12 | - | - |
| L14 | Synthetic waste with 100 gm/l Army Type I detergent, filtered | 2678 | 54,000 |

TABLE IA: ( Continued )

| Sample Number | Type of Sample | TOC mg/l | TS mg/l |
|---|---|---|---|
| L15 | Synthetic 500X, filtered | 72,000 | 248,000 |
| L16 | L15 diluted 1:1 | 42,000 | 130,000 |
| L17 | Mixture of L15 + S59 (equal volume of each) | 46,000 | 134,000 |
| L18 | Same as L14 but with 500 gm/l detergent, filtered | 75,000 | 183,000 |
| L19 | Mixture of L18 + S59 (equal volume of each) | 60,000 | 109,500 |
| L20 | Synthetic waste with 500 gm/l Army Type I detergent + vegetable oil, filtered | 77,500 | 230,000 |
| L21 | unfiltered L15 | 106,250 | 520,000 |
| L22 | L15 diluted 2:1 | 24,000 | - |
| L23 | L20 diluted 3:1 | 19,000 | - |
| L24 | Same as L15 | 72,000 | - |
| L25 | L24 diluted 1:1 | 36,500 | - |
| L26 | Synthetic 75X, filtered | 930 | |

End of Table IA.

TABLE IIA: Irritancy of the Specified Waste to the Eye of Rabbits.

| Type of Water | (N) | Source of Water, TOC (mg/L) and Number of Responses | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ultrafiltrate | | Feed Waste | | Concentrate | | Freeze Concentrate | |
| | | TOC mg/L | R(+) | TOC mg/L | (R+) | TOC mg/L | R(+) | TOC mg/L | R(+) |
| | | | | | ACTUAL SHOWER WASTES | | | | |
| 1X | 2 | | | 47 | 2/6 | | | 188 | 1/6 |
| | 2 | 25 | 1/6 | | | | | 147 | 0/6 |
| | 1 | | | (S-20) | | | | 340 | 1/6 |
| | 2 | | | S-61, 62 | | 1215 | 1/12 | | |
| | 8 | 19-68 | 0/24 | 72 | 0/6 | | | 202-337 | 0/18 |
| | 2 | | | (S-23, 51) | | | | 215-746 | 0/12 |
| | 2 | | | (S-48, 54) | | 104-116 | 0/12 | | |
| | 2 | | | (S-60, 51) | | 941-1426 | 0/12 | | |
| | | | | | SYNTHETIC SHOWER WASTES. | | | | |
| 1X | 6 | 26 | 0/6 | 55 | 2/6 | 141 | 0/12 | 367 | 0/6 |
| | 2 | (S-35) | | 30 | 1/6 | | | 215 | 0/6 |
| 5X | 1 | (S-22) | | | | | | 624 | 1/6 |
| 10X | 4 | 98 | 2/12 | 204 | 1/6 | | | | |
| | 2 | 49 | 1/6 | 210 | *4/6 | | | | |
| | 3 | 113 | 0/12 | (S-16) | | | | 1052 | 0/6 |
| | 1 | | | (S-39) | | | | 602 | 0/6 |
| 20X | (3) | | | 1030 | 1/12 | | | | |
| 30X | 4 | | | 1620 | 2/24 | | | | |
| 50X | (2) | | | 3093 | 2/12 | | | | |
| 100X | (1) | | | 3602 | *4/6 | | | | |
| | (1) | | | 3748 | 0/6 | | | | |
| | (1) | | | 6044 | 0/6 | | | | |
| 200X/4 | (1) | | | 1556 | 1/6 | | | | |
| 200X/2.5 | (1) | | | 2181 | 1/6 | | | | |
| 200X/2 | (1) | | | 2738 | 1/6 | | | | |
| 200X | (1) | | | 4942 | 1/6 | | | | |
| 1000X/8 | (1) | | | ? | 0/6 | | | | |
| 1000X/4 | (2) | | | 15000 | 3/12 | | | | |
| 1000X/2 | (1) | | | 28000 | 3/6 | | | | |
| 500X | (1) | | | 28161 | 0/6 | | | | |
| 1000X | (2) | | | 56000 | *10/12 | | | | |

TABLE IIA: ( Continued ).

| Type of Water | (N) | Source of Water, TOC (mg/L) and Number of Responses: Ultrafiltrate TOC mg/L | Ultrafiltrate R(+) | Feed Waste TOC mg/L | Feed Waste R(+) | Concentrate TOC mg/L | Concentrate R(+) | Freeze Concentrate TOC mg/L | Freeze Concentrate R(+) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | SYNTHETIC LAUNDRY WASTES | | | | | |
| 2X | (2) | 4 | 0/6 | 40 | 0/6 | | | 40 | 0/6 |
| 5X | (1) | 15 | 2/6 | | | | | | |
| 10X | (3) | 50 | 1/6 | 252 | 3/12;4/12 | | | | |
| 50X | (1) | | | 846 | 0/6 | | | | |
| 75X | (1) | | | 930 | 0/6 | | | | |
| 100X | (2) | | | 2643 | *8/12 | | | | |
| 500X/3 | (1) | | | 24000 | *4/6 | | | | |
| 500X/2 | (1) | | | 36501 | *5/6 | | | | |
| 500X | (2) | | | 72000 | *12/12 | | | | |

| | (N) | Type Formulation | TOC | R(+) |
|---|---|---|---|---|
| | | SPECIAL FORMULATIONS OF LAUNDRY WASTE | | |
| | (1) | 1X + 20g/l Neodol | 936 | 2/6 |
| | 1 | 1X + 20g/l AT1 | 986 | 0/6 |
| | 2 | 1X + 50g/l AT1 | 1843 | 0/12 |
| | 1 | 1X + 100g/l AT1 | 2678 | 0/6 |
| | 1 | (1X + 500g/l AT1)/4 | 19000 | 0/6 |
| | 2 | 1X + 500g/l AT1 | 75000 | 3/12 |
| | | MIXTURES OF EQUAL VOLUMES OF SHOWER AND LAUNDRY WASTE | | |
| | 1 | (500X + 500X)/2 | 46000 | 6/6 |
| | 1 | (500X + 1X + 500g/l AT1)/2 | 60000 | 0/6 |
| | | SPECIAL FORMULATIONS OF SHOWER WASTES | | |
| (S-27,28) | (2) | Na-ortho-phenyl phenolate (1000 mg/l) | 540 | 0/12 |
| (S-29) | 1 | Scouring cleaner (1000 mg/l) | 16 | 0/6 |

(*) Waste classified as an eye irritant by the test.
R(+) Number of positive responses/number tested.

TABLE IIIA: Informed Consent to Participate in a Medical Study.

Before agreeing to participate in this study it is important that the following explanation of the proposed procedures be read and understood. It describes the purpose, benefits, risks and precautions of the study. It also describes the alternatives available and the right to withdraw from the study at any time. It is important to understand that no guarantee or assurance can be made as to the results.

Objectives

I, _____, agree to participate in a medical study the purpose of which is to determine the potential for producing irritation of the skin of water which has been processed to remove waste materials (Such as detergents, tooth paste etc.). The samples will include Cincinnati tap water as well as other water.

Procedures

The study will use a standard technique, 21 day cumulative insult patch testing. Between four and eight small patches of gauze wetted with the water to be studied will be applied to the skin of my back every day for 3 weeks. The next day the patches will be removed, the skin observed and after a period of 30 minutes patches will be reapplied. During the period that patches are on my skin I should avoid prolonged immersion in water such as swimming or taking a bath. I will however be able to take short showers.

Risks and Precautions

The samples of water may produce mild irritation of the skin including redness and scaling confined to the area of application. This is considered unlikely because all samples will pass the chemical and biological standards for reuse shower water of the National Academy of Science. In addition all water samples will have been tested for their irritant effect on the skin of animals before they are used in this study.

If any significant irritation should occur from any water sample on my skin, further testing of that sample on me will be discontinued. In the unlikely case that it were necessary, appropriate dermatologic treatment, such as the application of a cream or ointment to such an area, would be given.

Availability of Information

I am free to ask additional questions regarding the study at any time.

The Right to Withdraw

I am free to withdraw from the study at any time, preferably, using a signed and witnessed statement such as this.

Volunteer _____ Date _____

Investigator _____ Date _____

Witness _____ Date _____

Witness _____ Date _____

## TABLE 1VA - Tabulation Form

Date:

From _____

TO _____

Name _____

| CONTACTANTS | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | | | | | | | | | | | | | | | | | | | | | |
| 2. | | | | | | | | | | | | | | | | | | | | | |
| 3. | | | | | | | | | | | | | | | | | | | | | |
| 4. | | | | | | | | | | | | | | | | | | | | | |
| 5. | | | | | | | | | | | | | | | | | | | | | |
| 6. | | | | | | | | | | | | | | | | | | | | | |
| 7. | | | | | | | | | | | | | | | | | | | | | |
| 8. | | | | | | | | | | | | | | | | | | | | | |

COMMENTS:

## APPENDIX B

A series of Progress Reports on the Toxicity and Irritancy of Ultrafiltrates of Non-sanitary Military Wastes which were presented periodically during the course of the study to the U.S. Army Medical Research and Development Command. They contain the detailed results obtained with each water.

PROGRESS REPORTS

# The Toxicity and Irritancy of Ultrafiltrates of Non-sanitary Military Wastes

Contract No. DAMD17-76-C-6006

First Quarterly Report, Fiscal Year 1976

From: Kettering Laboratory
Department of Environmental Health
University of Cincinnati
3223 Eden Avenue
Cincinnati, Ohio 45267

Approved:

Sylvan Witherup

Sylvan Witherup, B.S.
Assistant Professor of Toxicology
Principal Investigator

Paul B. Hammond

Paul B. Hammond, D.V.M., Ph.D.
Toxicology Division Head

Date: October 28, 1975

## Introduction

The University of Kentucky in contract with the U.S. Army Medical Research and Development Command (DADA17-72-C-2050) is developing membrane ultrafiltration as a waste treatment and water renovation for non-sanitary military wastes, specifically (a) laundry wastes, (b) shower wastes and (c) combined wastes containing both laundry and shower waste constituents. The components expected in the respective wastes have been described in the U.K. contract.

In this research, toxicity tests serve two purposes: First to detect changes in the toxic and irritant properties of the water which are occasioned by changing the treatment process; these tests must quantitate physiological effects in correlation with the efficiency of the treatment process. Secondly tests are needed also which will provide data suitable in part for judging the safety of water processed by a specific procedure. These toxicity tests are being performed in the Division of Toxicology, Department of Environmental Health, University of Cincinnati and are the subject of this report.

## Materials and Methods

Samples of water provided by the University of Kentucky have consisted of synthetic and real shower and laundry wastes before treatment, samples of the same water after specific treatment process and samples of the concentrated wastes remaining. To date, 31 samples have been received, of which 20 are included in this report. Shipped from Lexington, Kentucky via United Parcel Service they were received at Kettering the next morning. The samples were transferred from plastic containers to pyrex glass bottles, sealed and stored

in refrigeration until used in the experiments. Usually five consecutively numbered samples were taken from the refrigerated supply, identified by number only and studied without reference to the components or treatment process.

Male CF mice received in lots of 250 per week from Carworth Farms were distributed randomly, 5 per cage, among 50 cages contained on one rack.

Male New Zealand white rabbits weighing 2½ to 3½ kg were obtained weekly in lots of 24 from various local rabbitrys; these were caged individually and were observed with respect to their activities for 4 or 5 days prior to their use.

Oral Toxicity

After one week of observation with respect to their normal activity, 10 mice were weighed individually and given by intubation a dosage of 10,25, 40,63, or 100 ml per kg of a specific water; a total of 50 mice were given the respective dosages of any one water and 5 water samples were studied with each lot of mice. After dosage, the animals were observed daily during 14 days for signs of illness. All fatalities and any change in normal activity were noted in the records. The animals in each cage were weighed as a group on days 3,7, and 14 after their dosage.

Primary Irritation

The ability of each water to produce primary irritation in the skin was measured according to the patch test technique described in paragraph 191.1 of Regulations under the Federal Hazardous Substances Act Part 191, Chapter 1, Title 21, Code of the Federal Regulations first published by the U.S. Department of Health, Education, and Welfare, Food and Drug Administration , February 1965. 0.5 ml of the test water was placed on a cotton swab about one square inch in area and placed in contact with the bare abdominal skin; two such patches were used with each water, one placed upon intact skin and the other upon an area of abraded skin on each animal. The patches were covered with a plastic sheet

encircling the trunk and covered with a denim corset which kept the assembly in place and permitted the animal to be returned to its cage. After 24 hours, the coverings and patches were removed and the reactions in the skin were scored according to their severity as described in the procedural regulations summarized in Table 1.

Eye Irritation

The irritation produced in the eye following contact of the ocular tissues with a specific water was measured according to the definitive test described in paragraph 191.12 of the regulations cited above. Six rabbits were used for each test. Each eye was examined for redness and chemosis in the palpebral and bulbar conjunctivae and for any abnormality in the cornea or iris. If the ocular tissues were not normal in appearance the animal was excluded from the study. The test water was placed in one eye of each animal by gently pulling the lower lid away from the eye ball to form a cup into which 0.1 ml of the water was dropped. The lids were held together for one second and the animal released. The eyes were examined and the ocular reaction was recorded at 24,48 and 72 hours. Grades for scoring the ocular lesions as defined in the Regulations are shown in Table 3.

## Results

Primary Irritation

The average scores for erythema and for edema in the intact and abraded skin of 6 rabbits immediately following and on the 3rd day after 24 hours of skin contact with the respective waters are given in Table 4. The overall irritation score is obtained by adding the two sums (for erythema and edema) and dividing the total by 4 (two skin conditions and 2 time periods).

The skin reaction to any of the 20 samples listed in the tabulation consisted

of no more than a mild to moderate erythema with occasional mild edema. The maximum skin irritation score was 1.2 for sample S1.

The procedural regulations define a primary skin irritant as any substance which produces an empirical score of 5 or more when tested in this manner.

Eye Irritation

A summary of the irritant effects of the specific waters when placed in contact with the ocular tissues of rabbits is given in Table 5. There was no involvement of the cornea or the iris in any instance; the responses consisted of no more than slight to moderate erythema (redness) in the palpebral conjunctiva (without chemosis or swelling).

In the procedural regulations, eye irritancy is judged not by the average socre, but by the number of rabbits (in a group of 6) which show a given severity of any response (as indicated by the bracketed scores in Table 3) at any time during the 72 hours of observation. The number of animals showing a significant degree of erythema is indicated under N in the tabulation. Only water S6 was judged an eye irritant; the results with three waters (T8, L1 and L4) each with 3 animals showing a positive response were inconclusive and we will repeat the test with these samples. The total eye irritation score is provided in the tabulations as a basis for comparing the irritation potential of the respective waters.

Oral Toxicity

The data pertaining to the oral toxicity of the respective water samples are given in TAbles 6A,B,C, and D. An occasional small mouse became entangled in the wire mesh of the cage. If this occurred during the weekend, the animal was removed from the experiment and was killed, otherwise it was extricated and if uninjured was returned to the experiment. A few spontaneous deaths occurred in each lot of mice before the animals were dosed with the water assigned. An occasional death after a low dose of any one of the waters was believed also to result from extraneous disease unrelated to the water ingested. Such conclusion

is supported in part by no mortality among mice given much larger dosages of the same samples.

Changes in the body weight of the mice during two weeks post dosage observation are being evaluated for the statistical significance of differences with respect to dosage level and the water sample intubated; these computations have not been completed as yet. In general however, the body weights reveal no outstanding inhibition of growth attributable to any one of the specific waters.

Summary

Overall comparison of the several samples is provided in Table 7, a summary of the data for each water. In general the implications of the results to date are as follows:

1. None of the samples described in this report was considered a primary skin irritant as judged by the definitive tests of the Hazardous Substances Act. Each had a primary irritation score much smaller than the empirical score of 5 required to classify the water as such.

2. One sample, S4, was judged to be an irritant to the eye, the effect being limited to erythema in the palpebral conjunctiva. There was no edema in the conjunctiva and no injury in the cornea or iris. Three samples require additional study.

3. All of the water samples investigated to date were non-toxic when given orally to mice.

Table 1

List and Description of Water Samples Received to Date from the University of Kentucky for Toxicological Study

| Date Rec'd / Sample No. | Description of Sample |
|---|---|
| 7/30/75 | |
| S1 | Synthetic Shower Waste Feed Water |
| S2 | Synthetic Shower Waste Feed Water Concentrated |
| S3 | Synthetic Shower Waste Feed |
| S4 | Synthetic Shower Waste Feed Concentrated |
| S5 | Synthetic Shower Waste Ultrafiltrate |
| S6 | Synthetic Shower Waste Feed with Ajax Disinfectant DEET |
| S7 | Synthetic Shower Waste Ultrafiltrate (Minus Ajax Disinfectant DEET) |
| S8 | Synthetic Shower Waste Ultrafiltrate Chlorinated |
| T8 | Tap Water Concentrated |
| L1 | Synthetic Laundry Waste Feed |
| L2 | Synthetic Laundry Waste Ultrafiltrate |
| L3 | Composite Laundry Waste Ultrafiltrate from Long Run |
| L4 | Synthetic Laundry Waste Feed |
| 8/20/74 | |
| S9 | Shower Waste |
| S10 | Shower Waste |
| S11 | Shower Waste |
| S12 | Shower Waste |
| S13 | Shower Waste |
| S14 | Shower Waste |
| S15 | Shower Waste |
| S16 | Shower Waste |
| S17 | Shower Waste |
| L5 | Laundry Waste |
| L6 | Laundry Waste |
| 10/8/75 | |
| S18 | Synthetic Shower Waste Feed Filtered High Concentrated |
| S19 | Synthetic Shower Waste Feed Filtered Very High Concentrated |
| S19a | Synthetic Shower Waste Feed Filtered Very High Concentrated Chlorinated |
| S20 | Real Shower WAste Feed Chlorinated Filtered Concentrated |
| S21 | Concentrated Ultrafiltrate from real Shower Waste |
| S22 | Concentrated Ultrafiltrate from Synthetic Shower Waste |
| S23 | Concentrated Filtered Unchlorinated real Shower Waste Feed |

## TABLE 2

### SCORING PRIMARY IRRITATION

| ERYTHEMA AND ESCHAR FORMATION: | VALUE[1] |
|---|---|
| NO ERYTHEMA | 0 |
| VERY SLIGHT ERYTHEMA (EARELY PERCEPTIBLE) | 1 |
| WELL-DEFINED ERYTHEMA | 2 |
| MODERATE TO SEVERE ERYTHEMA | 3 |
| SEVERE ERYTHEMA (BEET REDNESS) TO SLIGHT ESCHAR FORMULATION (INJURIES IN DEPTH) | 4 |

| EDEMA FORMATION: | VALUE[1] |
|---|---|
| NO EDEMA | 0 |
| VERY SLIGHT EDEMA (BARELY PERCEPTIBLE) | 1 |
| SLIGHT EDEMA (EDGES OF AREA WELL DEFINED BY DEFINITE RAISING) | 2 |
| MODERATE EDEMA (RAISED APPROXIMATELY 1 MILLIMETER) | 3 |
| SEVERE EDEMA (RAISED MORE THAN 1 MILLIMETER AND EXTENDING BEYOND THE AREA OF EXPOSURE) | 4 |



READINGS ARE AGAIN MADE AT THE END OF A TOTAL OF 72 HOURS (48 HOURS AFTER THE FIRST READING). AN EQUAL NUMBER OF EXPOSURES ARE MADE ON AREAS OF SKIN THAT HAVE BEEN PREVIOUSLY ABRADED.

[1] THE "VALUE" RECORDED FOR EACH READING IS THE AVERAGE VALUE OF THE SIX OR MORE ANIMALS SUBJECT TO THE TEST.

Table 3

## Grades for Ocular Lesions

### Cornea

| | |
|---|---|
| No ulceration or opacity | 0 |
| Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible | (1)* |
| Easily discernible translucent areas, details of iris slightly obscured | 2 |
| Nacreous areas, no details of iris visible, size of pupil barely discernible | 3 |
| Complete corneal opacity, iris not discernible | 4 |

### Iris

| | |
|---|---|
| Normal | 0 |
| Markedly deepened folds, congestion, swelling, moderate circumcorneal injection (any of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive) | (1)* |
| No reaction to light, hemorrhage, gross destruction (any or all of these) | 2 |

### Conjunctivae

| | |
|---|---|
| *Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris).* | |
| Vessels normal | 0 |
| Some vessels definitely injected | 1 |
| Diffuse, crimson red, individual vessels not easily discernible | (2)* |
| Diffuse beefy red | 3 |
| *Chemosis* | |
| No swelling | 0 |
| Any swelling above normal (includes nictitating membrane) | 1 |
| Obvious swelling with partial eversion of lids | (2)* |
| Swelling with lids about half closed | 3 |
| Swelling with lids more than half closed | 4 |

*Bracketed figures indicate lowest grades considered positive under Section 191.12 of the Federal Hazardous Substances Labeling Act Regulations.

Table 4

Primary Irritation in the Skin of Rabbits

| Condition of the Skin | Time (hrs) | Average Score (6 Rabbits) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema |
| | | S1 | | S2 | | S3 | | S4 | |
| Intact | 24 | 1.0 | 0.3 | 0.5 | 0.7 | 0.5 | 0.7 | 0.5 | 0.3 |
| Abraded | 24 | 1.7 | 1.7 | 1.0 | 1.3 | 1.0 | 0.3 | 1.5 | 0.7 |
| Intact | 72 | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 0.0 | 0.0 | 0.0 |
| Abraded | 72 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | Sums | 2.7 | 2.0 | 1.5 | 2.0 | 1.7 | 1.0 | 2.0 | 1.0 |
| Irritation Score | | 1.2 | | 0.9 | | 0.7 | | 0.7 | |
| | | S5 | | S6 | | S7 | | S8 | |
| Intact | 24 | 1.2 | 0.5 | 1.2 | 0.5 | 0.3 | 0.2 | 0.2 | 0.0 |
| Abraded | 24 | 1.2 | 1.0 | 1.3 | 0.8 | 0.5 | 0.3 | 0.7 | 0.0 |
| Intact | 72 | 0.0 | 0.0 | 0.3 | 0.3 | 0.2 | 0.2 | 0.3 | 0.2 |
| Abraded | 72 | 0.2 | 0.0 | 0.0 | 0.0 | 0.3 | 0.2 | 0.5 | 0.0 |
| | Sums | 2.6 | 1.5 | 2.8 | 1.6 | 1.3 | 0.9 | 1.7 | 0.2 |
| Irritation Score | | 1.0 | | 1.1 | | 0.5 | | 0.5 | |

Table 4
Continued

| Condition of the Skin | Time (hrs) | Average Score (6 Rabbits) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema |
| | | L1 | | L2 | | L3 | | L4 | |
| Intact | 24 | 0.0 | 0.0 | 0.8 | 0.2 | 0.3 | 0.0 | 0.0 | 0.0 |
| Abraded | 24 | 1.7 | 0.5 | 1.2 | 0.3 | 1.2 | 0.3 | 1.7 | 0.8 |
| Intact | 72 | 0.0 | 0.0 | 0.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Abraded | 72 | 0.2 | 0.0 | 0.3 | 0.0 | 0.2 | 0.0 | 0.5 | 0.2 |
| Sums | | 1.9 | 0.5 | 2.6 | 0.5 | 1.7 | 0.3 | 2.2 | 1.0 |
| Irritation Score | | 0.6 | | 0.8 | | 0.5 | | 0.8 | |
| | | T8 | | S9 | | S10 | | S11 | |
| Intact | 24 | 0.7 | 0.2 | 0.3 | 0.0 | 0.3 | 0.0 | 0.0 | 0.0 |
| Abraded | 24 | 1.3 | 0.8 | 1.7 | 1.3 | 1.7 | 0.2 | 2.0 | 0.8 |
| Intact | 72 | 0.0 | 0.0 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Abraded | 72 | 0.0 | 0.0 | 0.7 | 0.2 | 0.7 | 0.0 | 0.7 | 0.5 |
| Sums | | 2.0 | 1.0 | 2.9 | 1.5 | 2.7 | 0.2 | 2.7 | 1.3 |
| Irritation Score | | 0.8 | | 1.1 | | 0.7 | | 1.0 | |

**Table 4**
**Continued**

| Condition of the Skin | Time (hrs) | Average Score (6 Rabbits) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema |
| | | S12 | | S13 | | S14 | | S15 | |
| Intact | 24 | 0.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Abraded | 24 | 1.5 | 0.2 | 1.5 | 0.8 | 1.0 | 0.0 | 1.3 | 0.3 |
| Intact | 72 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Abraded | 72 | 0.2 | 0.0 | 0.5 | 0.5 | 0.2 | 0.0 | 0.2 | 0.2 |
| Sum | | 2.0 | 0.2 | 2.0 | 1.3 | 1.2 | 0.0 | 1.5 | 0.5 |
| Irritation Score | | 0.6 | | 0.8 | | 0.3 | | 0.5 | |

**Table 4**
**Continued**

| Condition of the Skin | Time (hrs) | Average Score (6 Rabbits) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema |
| | | S12 | | S13 | | S14 | | S15 | |
| Intact | 24 | 0.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Abraded | 24 | 1.5 | 0.2 | 1.5 | 0.8 | 1.0 | 0.0 | 1.3 | 0.3 |
| Intact | 72 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Abraded | 72 | 0.2 | 0.0 | 0.5 | 0.5 | 0.2 | 0.0 | 0.2 | 0.2 |
| Sum | | 2.0 | 0.2 | 2.0 | 1.3 | 1.2 | 0.0 | 1.5 | 0.5 |
| Irritation Score | | 0.6 | | 0.8 | | 0.3 | | 0.5 | |

Table 5

Erythema Resulting When the Specified Water Was Placed In Contact With the Ocular Tissues In Rabbits (CF Table 3)

| Sample | Erythema in the Conjunctiva (Sum of 6) | | | | Number of Rabbits Judged Positive |
|---|---|---|---|---|---|
| | Lapsed Hours | | | Total Score | |
| | 24 | 48 | 72 | | |
| S1 | 5 | 3 | 6 | 14 | 2 |
| S2 | 4 | 3 | 1 | 8 | |
| S3 | 3 | 4 | 1 | 8 | 1 |
| S4 | 3 | 3 | 0 | 6 | |
| S5 | 5 | 3 | 3 | 11 | 2 |
| S6 | 3 | 4 | 6 | 13 | 4 (a) |
| S7 | 1 | 5 | 1 | 7 | 1 |
| S8 | 0 | 4 | 2 | 6 | |
| T8 | 7 | 3 | 4 | 14 | 3 (b) |
| L1 | 8 | 2 | 2 | 12 | 3 (b) |
| L2 | 4 | 1 | 1 | 6 | 1 |
| L3 | 6 | 3 | 3 | 12 | 2 |
| L4 | 7 | 3 | 5 | 15 | 3 (b) |
| S9 | 6 | 4 | 3 | 13 | 2 |
| S10 | 6 | 4 | 2 | 12 | 1 |
| S11 | 6 | 7 | 1 | 14 | 1 |
| S12 | 2 | 1 | 1 | 4 | |
| S13 | 3 | 2 | 1 | 6 | |
| S14 | 1 | 2 | 3 | 6 | |
| S15 | 1 | 2 | 4 | 7 | |

(a) Sufficient to classify the water as an eye irritant.

(b) Water as an eye irritant uncertain; additional animals required for classification.

Table 6 A

The Immediate Toxicity of Specific Water Samples When Given Orally to Mice. (CF Table 1 for description of samples; mice from Lot 1)

| Sample & Dosage ml/kg | Deaths (10 mice per dose) | Average Body Weight (g) 0 | 3 | 7 | 14 | Change in Weight (percent) |
|---|---|---|---|---|---|---|
| S1 | | | | | | |
| 10 | (1 k) | 14.3 | 16.8 | 19.4 | 23.2 | 62 |
| 25 | | 15.8 | 17.0 | 20.4 | 24.5 | 55 |
| 40 | (1 k) | 16.2 | 17.0 | 20.1 | 23.9 | 48 |
| 63 | | 17.8 | 20.0 | 21.6 | 25.1 | 41 |
| 100 | | 15.5 | 17.0 | 19.6 | 23.8 | 54 |
| Average | | 15.9 | 17.6 | 20.2 | 24.1 | 51 |
| S2 | | | | | | |
| 10 | 2 (7 days) | 13.6 | 15.9 | 16.3 | 22.4 | 80 |
| 25 | | 16.3 | 17.5 | 20.2 | 24.0 | 47 |
| 40 | | 16.8 | 18.7 | 21.0 | 25.4 | 51 |
| 63 | | 15.1 | 18.0 | 19.9 | 23.8 | 58 |
| 100 | | 15.7 | 17.4 | 20.2 | 23.4 | 49 |
| Average | | 15.5 | 17.5 | 19.7 | 23.9 | 54 |
| S3 | | | | | | |
| 10 | | 14.5 | 17.2 | 18.6 | 23.1 | 59 |
| 25 | | 15.2 | 16.8 | 19.8 | 23.3 | 53 |
| 40 | | 16.6 | 18.0 | 20.8 | 25.4 | 53 |
| 63 | | 16.4 | 17.9 | 20.2 | 24.3 | 48 |
| 100 | | 15.9 | 17.8 | 20.8 | 24.2 | 52 |
| Average | | 15.7 | 17.5 | 20.0 | 24.1 | 54 |
| S4 | | | | | | |
| 10 | 1 (6 days) | 14.2 | 17.5 | 17.8 | 20.9 | 47 |
| 25 | | 15.4 | 17.7 | 20.7 | 24.2 | 57 |
| 40 | | 15.7 | 17.3 | 20.6 | 24.2 | 54 |
| 63 | | 15.1 | 17.0 | 17.8 | 23.2 | 54 |
| 100 | | 15.3 | 16.0 | 18.9 | 22.6 | 48 |
| Average | | 15.1 | 17.1 | 19.9 | 23.1 | 53 |
| S5 | | | | | | |
| 10 | | 15.1 | 18.4 | 20.9 | 23.9 | 58 |
| 25 | | 16.6 | 19.7 | 21.6 | 23.9 | 44 |
| 40 | | 18.3 | 20.9 | 22.0 | 24.2 | 32 |
| 63 | | 18.3 | 20.4 | 23.3 | 24.7 | 35 |
| 100 | 1 | 17,7 | 19.2 | 22.2 | 25.1 | 42 |
| Average | | 17.2 | 19.7 | 21.8 | 24.3 | 42 |

(k) Accidentally killed.

**Table 6 B**

**The Immediate Toxicity of Specific Water Samples When Given Orally to Mice. (CF Table 1 for description of samples; mice from Lot 2)**

| Sample & Dosage ml/kg | Deaths (10 mice per dose) | Average Body Weight (g) 0 | 3 | 7 | 14 | Change in Weight (percent) |
|---|---|---|---|---|---|---|
| S6 | | | | | | |
| 10 | | 18.2 | 22.3 | 24.6 | 25.6 | 40.6 |
| 25 | | 17.5 | 21.3 | 22.3 | 23.4 | 33.7 |
| 40 | | 19.8 | 22.4 | 23.9 | 25.1 | 26.8 |
| 63 | | 19.5 | 23.1 | 23.2 | 24.0 | 23.1 |
| 100 | | 19.1 | 23.3 | 23.1 | 24.3 | 27.2 |
| Average | | 18.8 | 22.5 | 23.4 | 24.5 | 30.2 |
| S7 | | | | | | |
| 10 | 1 (2 days) | 18.3 | 20.0 | 23.0 | 26.0 | 42.1 |
| 25 | | 17.8 | 20.8 | 21.6 | 23.6 | 32.6 |
| 40 | | 18.7 | 21.6 | 23.2 | 24.8 | 32.6 |
| 63 | | 20.1 | 24.0 | 24.1 | 26.0 | 29.3 |
| 100 | | 18.2 | 21.2 | 22.8 | 24.0 | 31.9 |
| Average | | 18.6 | 21.6 | 22.9 | 24.9 | 33.6 |
| S8 | | | | | | |
| 10 | | 16.6 | 20.1 | 21.8 | 23.8 | 43.4 |
| 25 | | 19.2 | 21.1 | 23.3 | 25.9 | 34.9 |
| 40 | | 18.6 | 21.4 | 22.1 | 23.8 | 28.0 |
| 63 | | 19.5 | 22.7 | 23.2 | 23.6 | 21.0 |
| 100 | | 20.3 | 22.1 | 22.0 | 23.5 | 15.8 |
| Average | | 18.8 | 21.5 | 22.5 | 24.1 | 28.3 |
| T8 | | | | | | |
| 10 | | 17.1 | 20.3 | 21.5 | 23.5 | 37.4 |
| 25 | | 18.6 | 21.1 | 22.6 | 24.6 | 32.2 |
| 40 | | 18.1 | 17.2* | 20.2 | 22.9 | 26.5 |
| 63 | | 19.7 | 22.6 | 23.0 | 25.1 | 27.4 |
| 100 | (1 k) | 18.0 | 21.8 | 22.4 | 24.3 | 35.0 |
| Average | | 18.3 | 20.6 | 21.9 | 24.1 | 31.7 |
| L1 | | | | | | |
| 10 | | 18.7 | 20.7 | 22.6 | 24.5 | 31.0 |
| 25 | | 19.1 | 21.5 | 22.4 | 23.9 | 25.1 |
| 40 | | 18.6 | 21.6 | 22.4 | 22.7 | 22.0 |
| 63 | | 18.9 | 21.9 | 23.5 | 25.1 | 32.8 |
| 100 | | 19.4 | 22.0 | 21.1 | 22.7 | 17.0 |
| Average | | 18.9 | 21.5 | 22.4 | 23.8 | 25.8 |

*Five mice escaped from their cage and had no access to food; they were recaged and fed. Weight loss due to lack of food.

(k)Accidentally killed.

Table 6 C

The Immediate Toxicity of Specific Water Samples When Given Orally to Mice. (CF Table 1 for description of samples; mice from Lot 3)

| Sample & Dosage ml/kg | Deaths (10 mice per dose) | Average Body Weight (g) 0 | 3 | 7 | 14 | Change in Weight (percent) |
|---|---|---|---|---|---|---|
| L2 | | | | | | |
| 10 | | 15.5 | 16.8 | 19.7 | 21.3 | 37.4 |
| 25 | | 20.6 | 21.3 | 23.5 | 24.4 | 18.4 |
| 40 | | 18.9 | 18.9 | 20.4 | 21.2 | 12.2 |
| 63 | | 19.8 | 20.7 | 23.0 | 25.4 | 28.3 |
| 100 | 1 (2 days) | 19.5 | 20.7 | 23.2 | 24.9 | 27.7 |
| Average | | 18.9 | 19.6 | 21.9 | 23.4 | 23.8 |
| L3 | | | | | | |
| 10 | | 16.8 | 17.9 | 19.5 | 21.1 | 25.6 |
| 25 | | 18.9 | 19.8 | 22.4 | 24.2 | 28.0 |
| 40 | | 18.3 | 19.4 | 21.6 | 22.6 | 23.5 |
| 63 | | 20.5 | 21.1 | 23.1 | 24.9 | 21.5 |
| 100 | 1 (12 days) | 18.5 | 18.3 | 20.3 | 22.6 | 22.2 |
| Average | | 18.6 | 19.3 | 21.4 | 23.1 | 24.1 |
| L4 | | | | | | |
| 10 | | 16.3 | 17.8 | 20.1 | 20.9 | 28.2 |
| 25 | | 18.4 | 20.1 | 22.2 | 23.8 | 29.3 |
| 40 | 1 (6 days) | 20.1 | 19.9 | 22.2 | 22.8 | 13.4 |
| 63 | | 19.5 | 20.3 | 22.8 | 24.5 | 25.6 |
| 100 | | 20.1 | 20.2 | 21.6 | 22.6 | 12.4 |
| Average | | 18.9 | 19.6 | 21.8 | 22.9 | 21.1 |
| S9 | | | | | | |
| 10 | | 16.5 | 17.5 | 20.2 | 20.9 | 26.7 |
| 25 | | 19.6 | 20.3 | 22.4 | 23.8 | 21.4 |
| 40 | | 17.8 | 18.3 | 20.9 | 22.7 | 27.5 |
| 63 | 1 (10 days) | 25.0 | 23.9 | 24.8 | 26.7 | 6.8 |
| 100 | | 18.2 | 18.2 | 20.3 | 21.2 | 16.5 |
| Average | | 19.4 | 19.6 | 21.7 | 23.0 | 18.3 |
| S10 | | | | | | |
| 10 | | 20.2 | 22.5 | 23.9 | 23.1 | 14.4 |
| 25 | | 20.1 | 21.3 | 22.5 | 22.0 | 9.4 |
| 40 | | 24.5 | 23.2 | 27.0 | 27.0 | 10.2 |
| 63 | | 24.1 | 25.5 | 27.2 | 26.8 | 11.2 |
| 100 | 2 (3 days) | 23.4 | 24.0 | 26.1 | 27.1 | 15.8 |
| Average | | 22.8 | 23.4 | 25.5 | 25.4 | 11.4 |

Table 6 D

The Immediate Toxicity of Specific Water Samples When Given Orally to Mice. (CF Table 1 for description of samples; mice from Lot 4)

| Sample & Dosage ml/kg | Deaths (10 mice per dose) | Average Body Weight (g) 0 | 3 | 7 | 14 | Change in Weight (percent) |
|---|---|---|---|---|---|---|
| S11 | | | | | | |
| 10 | | 18.8 | 18.3 | 18.7 | 23.3 | 23.9 |
| 25 | | 19.0 | 18.5 | 17.1 | 22.7 | 19.5 |
| 40 | | 19.7 | 19.2 | 17.8 | 22.0 | 11.7 |
| 63 | 1 (3 days) | 18.5 | 19.3 | 20.2 | 22.8 | 23.2 |
| 100 | | 18.0 | 18.7 | 18.4 | 20.4 | 13.3 |
| Average | | 18.8 | 18.8 | 18.4 | 22.2 | 18.0 |
| S12 | | | | | | |
| 10 | | 19.2 | 19.7 | 21.1 | 23.6 | 22.9 |
| 25 | | 19.2 | 20.1 | 20.4 | 23.1 | 20.3 |
| 40 | 1 (5 days) | 18.6 | 21.0 | 21.7 | 25.1 | 34.9 |
| 63 | | 19.4 | 21.4 | 21.2 | 24.1 | 24.2 |
| 100 | | 20.5 | 22.6 | 20.5 | 25.1 | 22.4 |
| Average | | 19.4 | 21.0 | 20.5 | 23.7 | 22.0 |
| S13 | | | | | | |
| 10 | | 19.3 | 20.6 | 20.8 | 24.2 | 25.4 |
| 25 | | 18.9 | 19.7 | 19.4 | 22.7 | 20.1 |
| 40 | 1 (7 days) | 19.1 | 20.6 | 20.4 | 24.3 | 27.2 |
| 63 | | 19.4 | 21.6 | 21.4 | 23.1 | 19.1 |
| 100 | | 18.8 | 20.2 | 20.5 | 23.8 | 26.6 |
| Average | | 19.1 | 20.5 | 20.5 | 23.6 | 23.5 |
| S14 | | | | | | |
| 10 | | 18.3 | 19.3 | 21.4 | 23.5 | 28.4 |
| 25 | | 18.0 | 19.6 | 18.8 | 24.2 | 34.4 |
| 40 | | 20.2 | 20.9 | 21.1 | 24.0 | 18.8 |
| 63 | | 20.0 | 21.2 | 21.5 | 24.3 | 21.5 |
| 100 | | 18.6 | 18.9 | 18.1 | 22.2 | 19.4 |
| Average | | 19.0 | 19.9 | 20.1 | 23.6 | 24.3 |
| S15 | | | | | | |
| 10 | | 19.0 | 20.5 | 21.4 | 24.0 | 26.3 |
| 25 | | 19.4 | 20.8 | 20.5 | 23.8 | 22.7 |
| 40 | | 18.8 | 20.3 | 20.7 | 24.8 | 31.9 |
| 63 | 1 (12 days) | 19.9 | 19.3 | 18.8 | 24.5 | 23.1 |
| 100 | | 19.7 | 20.4 | 19.0 | 23.8 | 20.8 |
| Average | | 19.4 | 20.2 | 20.1 | 24.2 | 24.5 |

Table 7

Summary of Results

| Water Ident. Number | Irritation Scores | | | Oral Toxicity in Mice | | | | |
|---|---|---|---|---|---|---|---|---|
| | Eye | | Skin Aver. | Mortality 100 ml/kg | Average Wt (g) all doses (days after dose) | | | |
| | Sum | N* | Score | 10 mice | 0 | 3 | 7 | 14 |
| S1 | 14 | (2) | 1.2 | | 15.9 | 17.6 | 20.2 | 24.1 |
| S2 | 8 | | 0.9 | | 15.5 | 17.5 | 19.7 | 23.9 |
| S3 | 8 | (1) | 0.7 | | 15.7 | 17.5 | 20.0 | 24.1 |
| S4 | 6 | | 0.7 | | 15.1 | 17.1 | 19.9 | 23.1 |
| S5 | 11 | (2) | 1.0 | 1 (6 days) | 17.2 | 19.7 | 21.8 | 24.3 |
| S6 | 13 | (4) | 1.1 | | 18.8 | 22.5 | 23.4 | 24.5 |
| S7 | 7 | (1) | 0.5 | | 18.6 | 21.6 | 22.9 | 24.9 |
| S8 | 6 | | 0.5 | | 18.8 | 21.5 | 22.5 | 24.1 |
| T8 | 14 | (3) | 0.8 | 1(k)-6 days) | 18.3 | 20.6 | 21.9 | 24.1 |
| L1 | 12 | (3) | 0.6 | | 18.9 | 21.5 | 22.4 | 23.8 |
| L2 | 6 | (1) | 0.8 | | 18.9 | 19.6 | 21.9 | 23.4 |
| L3 | 12 | (2) | 0.5 | 1 (12 days) | 18.6 | 19.3 | 21.4 | 23.1 |
| L4 | 15 | (3) | 0.8 | | 18.9 | 19.6 | 21.8 | 22.9 |
| S9 | 13 | (2) | 1.1 | | 19.4 | 19.6 | 21.7 | 23.0 |
| S10 | 12 | (1) | 0.7 | 2 (3 days) | 22.8 | 23.4 | 25.5 | 25.4 |
| S11 | 14 | (1) | 1.0 | | 18.8 | 18.8 | 18.4 | 22.2 |
| S12 | 4 | | 0.6 | | 19.4 | 21.0 | 20.5 | 23.7 |
| S13 | 6 | | 0.8 | | 19.1 | 20.5 | 20.5 | 23.6 |
| S14 | 6 | | 0.3 | | 19.0 | 19.9 | 20.1 | 23.6 |
| S15 | 7 | | 0.5 | | 19.4 | 20.2 | 20.1 | 24.2 |

*Number of rabbits with a redness score of 2 (a definitive score). There was no effect on the cornea or iris.

(k) Killed accidentally.

AD _____

# THE TOXICITY AND IRRITANCY OF ULTRAFILTRATES OF NON-SANITARY MILITARY WASTES

Progress Report for November, 1975

Sylvan Witherup, B.S.

November 30, 1975

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Washington, D.C. 20314

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Department of Environmental Health
University of Cincinnati College of Medicine
Cincinnati, Ohio 45267

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## Introduction

This report contains data pertaining to the immediate oral toxicity and the irritant effects of ten water samples described in Table 1 and which continue in consecutive order from those described in the Quarterly Report dated October 28, 1975. It also includes a repeat of the eye irritation studies with samples L1 and L4. The experimental methods employed and the definitions of the empirical scores used in the irritation studies all have been described in the Quarterly Report.

## Results

### Skin Irritation

The average scores for erythema and for edema in the intact and abraded abdominal skin on six rabbits, immediately following and on the 3rd day after 24 hours of skin contact with the respective waters are given in Table 2. These water samples were somewhat more irritating to the skin of rabbits than were the preceding samples. The empirical scores ranged from 2.1 (Sample S 19) down to 0.6 (Sample S 17). The responses consisted of mild or moderate erythema and occasional mild edema which subsided rapidly in the intact skin and persisted but slightly longer in the abraded areas of skin. The scores, although somewhat higher than those obtained with the previous samples, were in all instances well below the empirical score of 5 required to classify a material as a primary skin irritant.

Eye Irritation

A summary of the eye irritation scores is provided in Table 3. None of the samples elicited sufficient response to be classified as an eye irritant. Two of the samples, (S 19a and S 20) resulted in a mild edema in the palpebrum which had not occurred previously. There was no involvement of the iris or cornea in any instance.

It had been noted previously that 3 of 6 rabbits had exhibited responses of sufficient severity to be considered positive after contact of the eyes with sample L1 or L4. In a repeat test no rabbit exhibited sufficient response to L1 to be judged positive by the standards, this water is not considered an eye irritant. One of 6 rabbits responded positively to L4. Although a 3rd test is necessary to classify the water as an irritant to the eye, I believe that for the purposes of these experiments, the data from the two experiments suffice and that the water should be considered irritating to the eye. It is noteworthy that in each instance the total scores for the two experiments were almost identical.

Oral Toxicity

Data pertaining to the oral toxicity of the respective waters are summarized in Tables 4A and 4B. As with the previous waters, these specific samples were not toxic when given orally to mice in dosages ranging from 10 to 100 ml/kg inclusively. Occasional deaths among the mice of Lot 5 were not dose related and were attributed to extraneous diseases. Diarrhea was noted in mice given 63 or 100 ml/kg of water S 18; a sanguineous excrement seen on the rear quarters and tail of some of the mice was not identified as to its source or contents.

Terminally the body weights of the mice were normal and not notably affected by any one of the waters.

## Summary

Ten water samples have been tested with respect to their irritancy and oral toxicity using methods described previously.

The pertinent observations are summarized in Table 5. None was judged to be a primary skin irritant. None was judged to be an irritant to the eye. However, both the skin and eye irritation scores were among the high values of all the waters tested to date. None of the waters was toxic when given orally to mice.

Table 1

Identification of the Waters Studied

| | |
|---|---|
| L5 | Laundry Waste |
| L6 | Laundry Waste |
| S16 | Shower Waste |
| S17 | Shower Waste |
| S18 | Synthetic Shower Waste Feed, Filtered, High Concentrate |
| S19 | Synthetic Shower Waste Feed, Filtered, Very High Concentrate |
| S19a | Synthetic Shower Waste Feed, Filtered, Very High Conc. Chlorinated |
| S20 | Real Shower Waste Filtered, Chlorinated Concentrated |
| S21 | Real Shower Waste Ultrafiltrate, Concentrated |
| S22 | Synthetic Shower Waste, Ultrafiltrate Concentrated |

Table 2

Primary Irritation Induced in the Skin of Rabbits;
Scored According to Procedural Regulations

| Condition of the Skin | Time (hrs) | Average Score (6 Rabbits) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema |
| | | L5 | | L6 | | S16 | | S17 | |
| Intact | 24 | 0.2 | 0.0 | 0.7 | 0.0 | 0.8 | 0.2 | 0.2 | 0.0 |
| Abraded | 24 | 1.3 | 1.2 | 1.5 | 1.7 | 1.2 | 1.2 | 1.2 | 0.5 |
| Intact | 72 | 0.0 | 0.0 | 0.2 | 0.0 | 0.3 | 0.0 | 0.0 | 0.0 |
| Abraded | 72 | 0.8 | 0.3 | 0.7 | 0.5 | 0.7 | 0.2 | 0.3 | 0.2 |
| | Sums | 2.3 | 1.5 | 3.1 | 2.2 | 3.0 | 1.6 | 1.7 | 0.7 |
| Irritation Score | | 1.0 | | 1.3 | | 1.2 | | 0.6 | |
| | | S18 | | S19 | | S19a | | S20 | |
| Intact | 24 | 0.3 | 0.0 | 1.3 | 0.5 | 0.8 | 0.0 | 0.2 | 0.0 |
| Abraded | 24 | 1.8 | 1.2 | 2.2 | 1.7 | 2.0 | 1.5 | 2.0 | 1.3 |
| Intact | 72 | 0.0 | 0.0 | 0.5 | 0.0 | 0.2 | 0.0 | 0.0 | 0.0 |
| Abraded | 72 | 0.7 | 0.3 | 1.3 | 0.7 | 1.3 | 0.3 | 1.2 | 0.0 |
| | Sums | 2.8 | 1.5 | 5.3 | 2.9 | 4.3 | 1.8 | 3.4 | 1.3 |
| Irritation Score | | 1.8 | | 2.1 | | 1.5 | | 1.2 | |

Table 2
Continued

| Condition of the Skin | Time (hrs) | Average Score (6 Rabbits) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema |
| | | S21 | | S22 | | | | | |
| Intact | 24 | 0.7 | 0.0 | 0.7 | 0.2 | - | - | - | - |
| Abraded | 24 | 2.0 | 1.5 | 2.0 | 1.8 | - | - | - | - |
| Intact | 72 | 0.3 | 0.0 | 0.3 | 0.0 | - | - | - | - |
| Abraded | 72 | 1.5 | 1.0 | 0.8 | 0.7 | - | - | - | - |
| | Sums | 4.5 | 2.5 | 3.8 | 2.7 | | | | |
| Irritation Score | | 1.8 | | 1.6 | | | | | |

Table 3

Eye Irritation Induced in Rabbits by Placing the Water in Contact with the Ocular Tissue; Responses Scored According to Procedural Regulations at the Times Specified

| Ident. No. of Sample | Lapsed Hours 24 R * C | 48 R * C | 72 R * C | Total Score | Number of Rabbits Judged Positive |
|---|---|---|---|---|---|
| L5 | 2 | 2 | 1 | 5 | 0 |
| L6 | 2 | 1 | 4 | 7 | 0 |
| S16 | 2 | 3 | 4 | 9 | 0 |
| S17 | 1 | 3 | 4 | 8 | 0 |
| S18 | 5 | 4 | 1 | 10 | 1 |
| S19 | 4 | 5 | 2 | 11 | 1 |
| S19a | 4 1 | 1 1 | 3 1 | 12 | 1 |
| S20 | 4 2 | 1 1 | 1 1 | 10 | 1 |
| S21 | 2 | 0 | 2 | 4 | 0 |
| S22 | 5 | 2 | 2 | 9 | 1 |
| | Repeat of Two Samples Studied Previously | | | | |
| L1 | 3 | 5 | 5 | 13 | 0 |
| L4 | 6 | 5 | 5 | 16 | 1 |

*R = redness; C = Chemosis (edema) in the palpebral conjunctiva. There was no noticeable effect in the cornea or iris.

Table 4 A

The Immediate Toxicity of Specific Water Samples When Given Orally to Mice. (Lot M5)

| Sample Dosage ml/kg | Deaths (10 mice per dose) | Average Body Weight (g) 0 | 3 | 7 | 14 | Change in Weight |
|---|---|---|---|---|---|---|
| S16 | | | | | | |
| 10 | | 13.7 | 14.4 | 17.0 | 22.6 | 68 |
| 25 | | 13.3 | | 20.0 | 23.8 | 79 |
| 40 | | 14.5 | | 20.2 | 24.1 | 66 |
| 63 | | 15.7 | | 19.6 | 24.6 | 57 |
| 100 | 2; (1-9 days) | 14.6 | | 18.1 | 24.2 | 66 |
| Average | | 14.4 | 14.4 | 18.8 | 23.8 | 66 |
| S17 | | | | | | |
| 10 | | 13.7 | 14.8 | 19.5 | 22.6 | 65 |
| 25 | 1; (6 days) | 15.3 | | 18.6 | 22.2 | 45 |
| 40 | | 13.6 | | 16.7 | 20.8 | 53 |
| 63 | | 12.9 | | 16.6 | 21.9 | 70 |
| 100 | | 15.8 | | 18.0 | 22.3 | 41 |
| Average | | 14.3 | 14.8 | 17.9 | 21.9 | 53 |
| L5 | | | | | | |
| 10 | 1(K-5 days) | 12.5 | 14.7 | 18.5 | 23.7 | 90 |
| 25 | | 15.6 | | 17.0 | 20.9 | 34 |
| 40 | 1 (8 days) | 15.6 | | 16.7 | 20.4 | 31 |
| 63 | | 15.5 | | 20.2 | 24.2 | 56 |
| 100 | | 15.2 | | 19.3 | 23.4 | 54 |
| Average | | 14.9 | 14.7 | 18.3 | 22.5 | 51 |
| L6 | | | | | | |
| 10 | *(12 days) | 13.4 | 16.0 | 17.6 | 20.5 | 53 |
| 25 | | 13.6 | | 18.4 | 24.2 | 78 |
| 40 | | 13.8 | | 20.6 | 26.2 | 90 |
| 63 | | 15.3 | | 20.2 | 23.9 | 56 |
| 100 | 1 (8 days) | 14.8 | | 18.8 | 20.8 | 40 |
| Average | | 14.2 | 16.0 | 19.2 | 23.3 | 64 |

*Two mice escaped; eliminated from the experiment weights on 14th day.

Table 4 B

The Immediate Toxicity of Specific Water Samples When Given Orally to Mice. (Lot M6)

| Sample Dosage ml/kg | Deaths (10 mice per dose) | Average Body Weight (g) 0 | 3 | 7 | 14 | Change in Weight (percent) |
|---|---|---|---|---|---|---|
| S18 | | | | | | |
| 10 | | 18.9 | 21.4 | 22.6 | 23.8 | 26 |
| 25 | | 17.6 | 19.1 | 21.6 | 23.4 | 33 |
| 40 | | 19.6 | 21.0 | 23.6 | 23.9 | 22 |
| 63 | | 19.8 | 21.8 | 21.9 | 23.0 | 16 |
| 100 | | 17.2 | 19.0 | 21.8 | 23.4 | 36 |
| Average | | 18.6 | 20.5 | 22.3 | 23.3 | 25 |
| S19 | | | | | | |
| 10 | | 15.5 | 17.4 | 19.6 | 21.9 | 41 |
| 25 | | 16.5 | 17.4 | 20.1 | 20.3 | 23 |
| 40 | | 16.1 | 18.4 | 21.1 | 22.6 | 40 |
| 63 | | 17.0 | 19.8 | 22.4 | 24.0 | 41 |
| 100 | | 15.6 | 17.4 | 20.9 | 23.0 | 44 |
| Average | | 16.1 | 18.1 | 20.8 | 22.3 | 38 |
| S19a | | | | | | |
| 10 | | 17.4 | 18.8 | 20.9 | 22.6 | 30 |
| 25 | | 20.3 | 22.4 | 23.5 | 24.7 | 22 |
| 40 | | 18.7 | 20.2 | 22.0 | 22.9 | 22 |
| 63 | | 19.0 | 19.4 | 21.4 | 23.0 | 21 |
| 100 | | 16.8 | 19.2 | 21.3 | 22.2 | 28 |
| Average | | 18.4 | 20.0 | 21.9 | 23.1 | 26 |
| S20 | | | | | | |
| 10 | | 15.7 | 17.5 | 20.7 | 22.5 | 43 |
| 25 | | 18.1 | 20.5 | 22.1 | 23.4 | 29 |
| 40 | | 18.5 | 18.4 | 21.4 | 22.8 | 23 |
| 63 | | 19.1 | 22.7 | 20.5 | 22.7 | 19 |
| 100 | | 17.7 | 19.7 | 20.7 | 23.0 | 30 |
| Average | | 17.8 | 19.7 | 21.0 | 22.8 | 28 |
| S21 | | | | | | |
| 10 | | 14.6 | 17.6 | 20.2 | 22.4 | 53 |
| 25 | | 18.6 | 21.2 | 22.5 | 23.1 | 24 |
| 40 | | 18.0 | 20.6 | 21.5 | 22.9 | 27 |
| 63 | | 20.5 | 23.3 | 25.0 | 25.5 | 24 |
| 100 | | 17.1 | 17.5 | 19.5 | 22.3 | 30 |
| Average | | 17.8 | 20.0 | 21.7 | 23.2 | 30 |

Table 5

Summary of Results

| Water Ident. Number | Irritation Scores | | | Oral Toxicity in Mice | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Eye Sum | Skin (N*) | Aver. Score | Mortality 100 ml/kg 10 mice | Average Wt (g) all doses (days after dose) 0 | 3 | 7 | 14 | Change in wt. (Percent) |
| L5 | 5 | | 1.0 | | 14.9 | | 18.3 | 22.5 | 51 |
| L6 | 7 | | 1.3 | 1 (8 days) | 14.2 | | 19.2 | 23.3 | 64 |
| S16 | 9 | | 1.2 | 2 (10 days) | 14.4 | | 18.8 | 23.8 | 66 |
| S17 | 8 | | 0.6 | | 14.3 | | 17.9 | 21.9 | 53 |
| SS18 | 10 | 1 | 1.8 | | 18.6 | 20.5 | 22.3 | 23.3 | 25 |
| S19 | 11 | 1 | 2.1 | | 16.1 | 18.1 | 20.8 | 22.3 | 38 |
| S19a | 12 | 1 | 1.5 | | 18.4 | 20.0 | 21.9 | 23.1 | 26 |
| S20 | 10 | 1 | 1.2 | | 17.8 | 19.7 | 21.0 | 22.8 | 28 |
| S21 | 4 | 0 | 1.8 | | 17.8 | 20.0 | 21.7 | 23.2 | 30 |
| S22 | 9 | 1 | 1.6 | Incomplete* | * * * * ** * * * * * * * * * | | | | |
| L1 | 13 | 0 | - | | | | | | |
| L4 | 16 | 1 | - | | | | | | |

Unclassified

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# THE TOXICITY AND IRRITANCY OF ULTRAFILTRATES OF NON-SANITARY MILITARY WASTES

Progress Report for December 31, 1975

Sylvan Witherup, B.S.

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Washington, D.C. 20314

Contract No. DAMD17-76-C-6006

Department of Environmental Health
University of Cincinnati College of Medicine
Cincinnati, Ohio 45267

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The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

Unclassified

## TABLE OF CONTENTS

## Introduction

This report contains data pertaining to the immediate oral toxicity and the irritant properties of twenty water samples described in Table 1 and which continue in consecutive order from those described in the November Progress Report dated November 30, 1975. It also includes a repetition of the eye irritation study with sample T8. The experimental methods employed and the definitions of the empirical scores used in the irritation studies all have been described in the Quarterly Report dated October 28, 1975.

## Results

### Skin Irritation

The average scores for erythema and for edema in the intact and abraded abdominal skin on six rabbits, immediately following and on the 3rd day after 24 hours of skin contact with the respective waters are given in Table 2. None of the samples was found to be a primary skin irritant as defined by the Procedural Regulations which require an empirical score of 5 or more for this classification.

The average skin irritation scores obtained with laundry water samples ranged from a high of 3.5 for sample L12, 3.4 for L13, 3.15 for L9 and 2.65 for L7 (these were concentrated samples) down to a low of 0.3 and 0.4 for waters L10 and L11. It should be noted that the high average scores obtained with the specified laundry waste concentrates represent moderate

to severe erythema and edema with ulceration and eschar formation in some of the animals; usually these injuries had not healed completely within the 3 days period of observation.

A skin irritation score of 2 resulting from water S26 was the highest obtained among the shower waste waters; the values generally were less than one.

Eye Irritation

The empirical scores by which the irritant effects of the respective waters on the eyes of rabbits were graded are listed in Table 3. In all instances the response consisted of some degree of erythema in the palpebral conjunctiva, without noticeable swelling of the tissue and with no injury in the cornea or iris. The severity and extent of the responses was sufficient to classify sample S26 and Sample L13 as an eye irritant. The responses to L13 are equivocal, since there was a positive response in 3 of the 6 rabbits tested. An additional test is needed to classify the water; however in consideration of the skin irritation resulting from this sample (Table 2) it should be considered an eye irritant until more tests are performed.

A repetition of the eye irritation test with water T8 indicated that this water was <u>not</u> an eye irritant as has been suggested by the equivocal results obtained in the first test.

Immediate Oral Toxicity

Data pertaining to the immediate toxicity of the respective waters when given orally to mice are summarized in Tables 4A,B,C and D. Death

occurred only among mice that were given 100 ml/kg of L7, L12 or L13, the respective mortalities being 1/10, 6/10 and 3/10. Mice that were given sublethal dosages of these three samples usually showed some degree of weight loss or reduced growth that was related to the magnitude of the dose.

## Summary

Of 12 shower waste water samples only S26, a concentrated synthetic waste feed was found to be an eye irritation; slight irritation in the skin was not sufficient to classify the product as a primary skin irritant. No deaths occurred among mice that were given orally, 100 ml of any one of the shower wastes per kg body weight.

Laundry waste water samples L12 and L13 were classified as eye irritants and were moderately irritating to the skin of rabbits, although the severity and extent of the injuries on the skin were not sufficient to classify either water as a primary skin irritation. A mortality of 10, 60, and 30 percent occurred respectively among mice that were given orally, 100 ml/kg of water L1, L12 or L13.

Of the 20 samples tested, S25, L7, L12 and L13 were exceptional in the extent of the responses they elicited.

**Table 1**

**Identification of the Waters Studied**

| | |
|---|---|
| S-22 | Concentrated Ultrafiltrate from Real Shower Waste. |
| S-23 | Concentrated Filtered Unchlorinated Real Shower Waste Feed. |
| S-24 | Synthetic Shower Waste Ultrafiltrate-Concentrated. |
| S-25 | Real Shower Waste Ultrafiltrate-Concentrated. |
| S-26 | Synthetic Shower Waste Feed-Concentrated. |
| S-27 | (OPSS) Concentrated Disinfectant. |
| S-28 | (OPSS) Concentrated Disinfectant. |
| S-29 | Concentrated Ajax Solution. |
| S-30 | Synthetic Shower Waste Concentrated Feed. |
| S-31 | Synthetic Shower Waste Concentrated Feed. |
| S-32 | Synthetic Shower Waste Concentrated Feed. |
| S-33 | Synthetic Shower Waste Concentrated Feed. |
| T-9 | Tap Water; Concentrated. |
| T-10 | Tap Water Concentrated. |
| L-7 | Synthetic Laundry Waste Feed Concentrated. |
| L-8 | U.S. Army Detergent; Concentrated. |
| L-9 | Concentrated Tide Detergent. |
| L-10 | Army Detergent Concentrated. |
| L-11 | Army Detergent Concentrated. |
| L-12 | Synthetic Laundry Waste Concentrated. |
| L-13 | Synthetic Laundry Waste Concentrated. |

Table 2

Primary Irritation Induced in the Skin of Rabbits;
Scored According to Procedural Regulations

| Condition of the Skin | Time (hrs) | Average Score (6 Rabbits) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema |
| | | S23 | | S24 | | S25 | | S26 | |
| Intact | 24 | 0.2 | 0.0 | 0.5 | 0.0 | 0.0 | 0.0 | 1.7 | 0.7 |
| Abraded | 24 | 1.3 | 0.2 | 1.0 | 0.0 | 0.7 | 0.3 | 1.7 | 1.2 |
| Intact | 72 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.8 | 0.0 |
| Abraded | 72 | 0.5 | 0.0 | 0.0 | 0.0 | 0.2 | 0.0 | 0.7 | 0.2 |
| | Sums | 2.2 | 0.2 | 1.5 | 0.0 | 0.9 | 0.3 | 5.9 | 2.1 |
| Irritation Score | | 0.6 | | 0.4 | | 0.3 | | 2.0 | |
| | | S27 | | S28 | | S29 | | L7 | |
| Intact | 24 | 0.0 | 0.0 | 0.2 | 0.0 | 0.2 | 0.0 | 2.2 | 1.0 |
| Abraded | 24 | 1.3 | 0.5 | 1.3 | 0.2 | 1.7 | 0.3 | 2.8 | 1.8 |
| Intact | 72 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.3 | 0.0 |
| Abraded | 72 | 0.0 | 0.0 | 0.2 | 0.0 | 0.2 | 0.0 | 1.8 | 0.7 |
| | Sums | 1.3 | 0.5 | 1.7 | 0.2 | 2.1 | 0.3 | 7.1 | 3.5 |
| Irritation Score | | 0.4 | | 0.5 | | 0.6 | | 2.6 | |

Table 2 (Continued)

Primary Irritation Induced in the Skin of Rabbits;
Scored According to Procedural Regulations

| Condition of the Skin | Time (hrs) | Average Score (6 Rabbits) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema |
| | | T9 | | T10 | | L8 | | L9 | |
| Intact | 24 | 0.2 | 0.0 | 0.2 | 0.0 | 0.3 | 0.0 | 2.0 | 1.8 |
| Abraded | 24 | 1.7 | 0.5 | 1.2 | 0.2 | 1.8 | 0.3 | 2.7 | 2.3 |
| Intact | 72 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.0 | 0.0 |
| Abraded | 72 | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 0.0 | 1.8 | 1.0 |
| | Sums | 1.9 | 0.5 | 1.4 | 0.2 | 2.3 | 0.3 | 7.5 | 5.1 |
| Irritation Score | | 0.6 | | 0.4 | | 0.6 | | 3.2 | |
| | | L10 | | L11 | | L12 | | L13 | |
| Intact | 24 | 0.5 | 0.0 | 0.3 | 0.0 | 1.8 | 1.5 | 1.5 | 1.0 |
| Abraded | 24 | 1.5 | 0.2 | 1.3 | 0.2 | 2.2 | 1.8 | 2.0 | 1.5 |
| Intact | 72 | 0.0 | 0.0 | 0.0 | 0.0 | 2.2 | 0.2 | 1.8 | 0.2 |
| Abraded | 72 | 0.0 | 0.0 | 0.0 | 0.0 | 3.5 | 1.0 | 3.5 | 2.0 |
| | Sums | 2.0 | 0.2 | 1.6 | 0.2 | 9.7 | 4.5 | 8.8 | 4.7 |
| Irritation Score | | 0.4 | | 0.3 | | 3.5 | | 3.4 | |

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THE TOXICITY AND IRRITANCY OF ULTRAFILTRATES OF NON-SANITARY MI--ETC(U)
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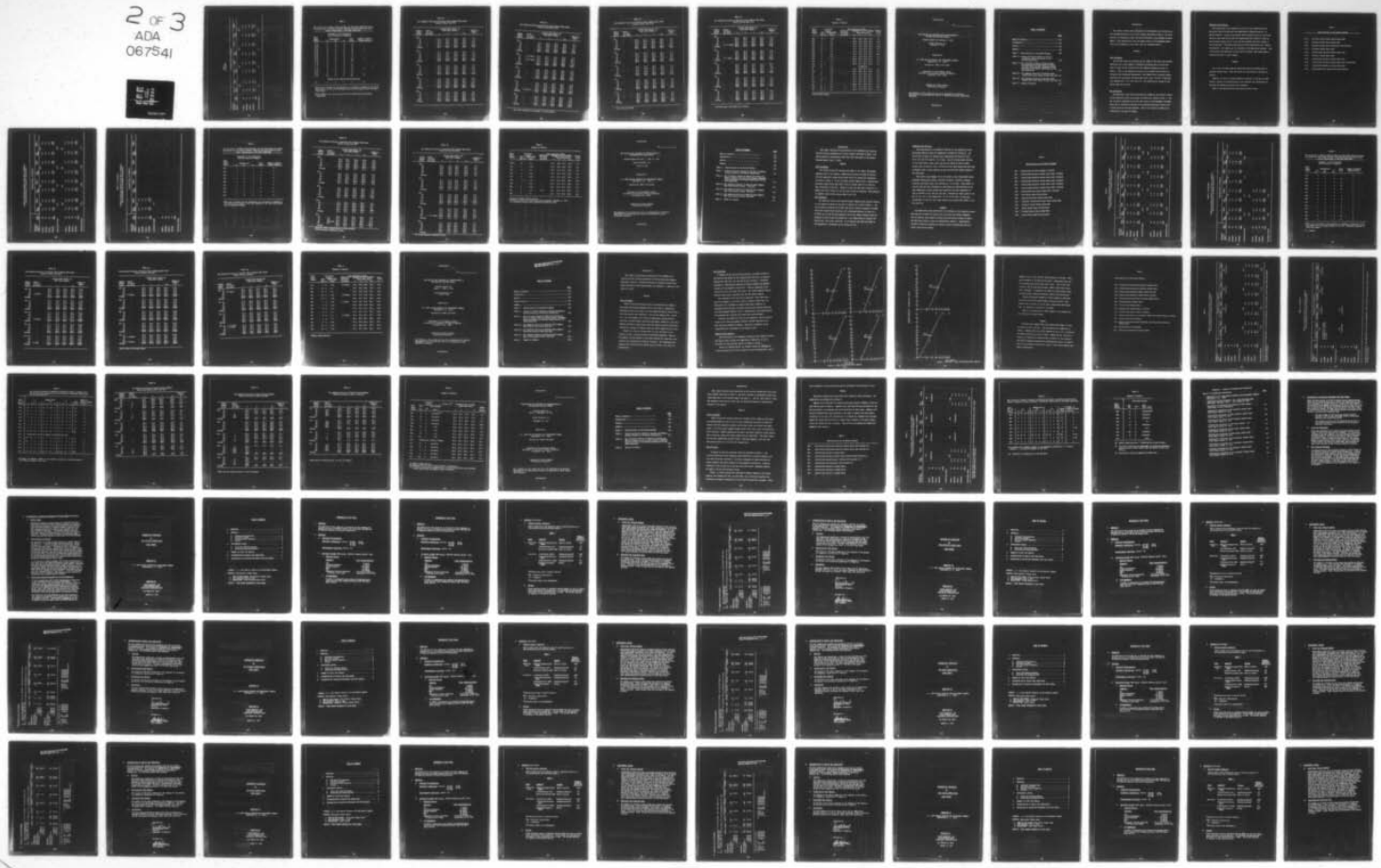

Table 2
Continued

| Condition of the Skin | Time (hrs) | Average Score (6 Rabbits) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema |
| | | S30 | | S31 | | S32 | | S33 | |
| Intact | 24 | 1.5 | 0.2 | 1.3 | 0.2 | 1.8 | 0.3 | 1.3 | 0.3 |
| Abraded | 24 | 1.8 | 0.5 | 2.0 | 0.7 | 2.2 | 1.0 | 2.3 | 1.2 |
| Intact | 72 | 0.0 | 0.0 | 0.0 | 0.0 | 0.7 | 0.0 | 0.5 | 0.0 |
| Abraded | 72 | 0.0 | 0.0 | 0.0 | 0.0 | 0.7 | 0.0 | 0.5 | 0.0 |
| | Sums | 3.3 | 0.7 | 3.3 | 0.9 | 5.4 | 1.3 | 4.6 | 1.5 |
| Irritation Score | | 1.0 | | 1.1 | | 1.7 | | 1.5 | |

Table 3

Eye Irritation in Rabbits Resulting When the Specified Water Was Placed in Contact with the Ocular Tissues; Responses Scored According to Procedural Regulations at the Times Specified

| Water Ident. Number | Erythema* in the Conjunctiva (Sum of Scores in 6 Rabbits) Lapsed Hours 24 | 48 | 72 | Total Score | Number of Rabbits Judged Positive |
|---|---|---|---|---|---|
| S 23 | 3 | 4 | 2 | 9 | |
| S 24 | 3 | 3 | 3 | 9 | |
| S 25 | 2 | 3 | 4 | 9 | |
| S 26 | 9 | 5 | 6 | 21 | 4 |
| S 27 | 2 | 3 | 3 | 8 | |
| S 28 | 4 | 4 | 1 | 9 | |
| S 29 | 4 | 3 | 2 | 9 | |
| S 30 | 2 | 1 | 2 | 5 | |
| S 31 | 3 | 2 | 1 | 6 | |
| S 32 | 5 | 4 | 2 | 11 | 2 |
| S 33 | 5 | 3 | 4 | 12 | |
| T 9 | 3 | 3 | 1 | 7 | |
| T 10 | 2 | 1 | 1 | 4 | |
| L 7 | 6 | 1 | 3 | 10 | |
| L 8 | 0 | 1 | 1 | 2 | |
| L 9 | 7 | 4 | 1 | 12 | 2 |
| L 10 | 1 | 2 | 2 | 5 | |
| L 11 | 4 | 1 | 0 | 5 | |
| L 12 | 8 | 4 | 1 | 14 | 3 |
| L 13 | 10 | 8 | 4 | 22 | 5 |
| Repeat of one sample studied previously | | | | | |
| T 8 | 0 | 1 | 1 | 2 | ** |

*There was no chemosis in the conjunctiva, no lesion or clouding of the cornea and no effect on the iris resulting at any time from contact with the specified waters.

**Water sample T 8 is not an eye irritant as defined by the Procedural Regulations.

Table 4A

The Immediate Toxicity of Specific Water Samples When Given Orally to Mice (Lot M 8)

| Sample Dosage ml/kg | Deaths (10 mice/ per dose) | Average Body Weight (g) (day after dosage) 0 | 3 | 7 | 14 | Change in Weight % |
|---|---|---|---|---|---|---|
| S22 | | | | | | |
| 10 | | 23.2 | 24.2 | 25.2 | 25.3 | 9.0 |
| 25 | | 24.0 | 23.5 | 26.0 | 25.3 | 5.2 |
| 40 | | 24.3 | 24.6 | 26.8 | 26.8 | 10.3 |
| 63 | | 22.7 | 22.3 | 22.6 | 21.2 | -6.6 |
| 100 | | 24.5 | 23.4 | 24.9 | 23.4 | -4.6 |
| Average | | 23.7 | 23.6 | 25.1 | 24.4 | 2.7 |
| S23 | | | | | | |
| 10 | | 24.3 | 24.6 | 26.1 | 26.6 | 9.7 |
| 25 | | 24.2 | 24.6 | 25.5 | 26.3 | 8.6 |
| 40 | | 22.8 | 22.7 | 24.6 | 24.7 | 8.4 |
| 63 | | 24.1 | 24.4 | 25.7 | 24.5 | 1.5 |
| 100 | | 22.5 | 21.9 | 22.3 | 20.7 | -8.3 |
| Average | | 23.6 | 23.6 | 25.0 | 24.5 | 4.1 |
| S24 | | | | | | |
| 10 | | 22.5 | 22.4 | 23.6 | 24.2 | 7.5 |
| 25 | | 23.2 | 24.0 | 26.3 | 26.3 | 13.6 |
| 40 | | 24.6 | 24.9 | 26.0 | 25.2 | 2.6 |
| 63 | | 23.7 | 23.8 | 25.2 | 25.4 | 7.4 |
| 100 | | 23.6 | 22.9 | 24.8 | 24.9 | 5.6 |
| Average | | 23.5 | 23.6 | 25.1 | 25.2 | 7.3 |
| S25 | | | | | | |
| 10 | | 23.4 | 23.8 | 24.9 | 26.6 | 13.8 |
| 25 | | 23.5 | 23.6 | 25.1 | 25.3 | 7.5 |
| 40 | | 23.9 | 24.2 | 26.4 | 26.4 | 10.3 |
| 63 | | 23.2 | 23.9 | 26.4 | 27.0 | 16.4 |
| 100 | | 22.5 | 23.1 | 24.8 | 26.2 | 16.3 |
| Average | | 23.3 | 23.7 | 25.5 | 26.3 | 12.8 |
| S26 | | | | | | |
| 10 | | 24.8 | 24.8 | 25.8 | 26.4 | 6.5 |
| 25 | | 23.8 | 24.2 | 25.8 | 26.2 | 9.7 |
| 40 | | 22.7 | 22.1 | 24.4 | 25.3 | 11.4 |
| 63 | | 24.1 | 22.2 | 24.8 | 25.8 | 4.2 |
| 100 | | 22.6 | 21.4 | 23.5 | 24.3 | 7.5 |
| Average | | 23.6 | 22.9 | 24.9 | 25.6 | 8.4 |

Table 4B

The Immediate Toxicity of Specific Water Samples When Given Orally to Mice (Lot M 9)

| Sample Dosage ml/kg | Deaths (10 mice/ per dose) | Average Body Weight (g) (day after dosage) 0 | 3 | 7 | 15 | Change in Weight % |
|---|---|---|---|---|---|---|
| S27 | | | | | | |
| 10 | | 22.6 | 22.5 | 25.3 | 24.4 | 8.2 |
| 25 | | 22.7 | 19.8 | 21.9 | 23.4 | 3.2 |
| 40 | | 24.1 | 24.4 | 24.9 | 25.6 | 6.3 |
| 63 | | 21.9 | 24.6 | 25.4 | 24.5 | 11.7 |
| 100 | | 25.2 | 25.2 | 25.1 | 26.6 | 5.8 |
| Average | | 23.3 | 23.3 | 24.1 | 24.9 | 7.0 |
| S28 | | | | | | |
| 10 | 1 (14 days) | 22.6 | 21.7 | 22.6 | 22.9 | 1.4 |
| 25 | | 21.4 | 23.0 | 23.1 | 24.8 | 16.0 |
| 40 | 1 (14 days) | 19.1 | 22.9 | 22.4 | 24.3 | 27.7 |
| 63 | | 22.8 | 24.4 | 25.2 | 26.7 | 17.2 |
| 100 | | 23.9 | 24.8 | 25.2 | 26.2 | 9.7 |
| Average | | 22.0 | 23.4 | 23.7 | 25.1 | 14.2 |
| S29 | | | | | | |
| 10 | | 24.8 | 24.8 | 24.7 | 23.4 | -5.5 |
| 25 | | 21.9 | 22.8 | 23.2 | 24.2 | 10.7 |
| 40 | * | 22.5 | 23.6 | 24.2 | 24.3 | 8.0 |
| 63 | * | 23.5 | 24.2 | 25.1 | 25.5 | 8.4 |
| 100 | | 24.0 | 25.0 | 26.0 | 27.4 | 14.1 |
| Average | | 23.3 | 24.1 | 24.6 | 25.0 | 4.2 |
| L7 | | | | | | |
| 10 | | 23.5 | 23.3 | 23.8 | 25.4 | 7.8 |
| 25 | | 22.8 | 24.4 | 25.2 | 26.9 | 18.8 |
| 40 | | 21.2 | 21.8 | 22.4 | 23.8 | 12.6 |
| 63 | | 24.3 | 22.8 | 24.0 | 25.6 | 5.3 |
| 100 | 1 (6 days) | 21.3 | 22.2 | 22.8 | 24.5 | 14.9 |
| Average | | 22.6 | 22.9 | 23.8 | 25.2 | 11.5 |
| L8 | | | | | | |
| 10 | | 22.4 | 23.8 | 24.4 | 24.0 | 7.5 |
| 25 | | 21.4 | 23.6 | 23.9 | 23.2 | 8.2 |
| 40 | | 22.6 | 23.0 | 23.6 | 24.8 | 9.9 |
| 63 | | 21.6 | 22.4 | 23.1 | 24.4 | 12.9 |
| 100 | | 23.1 | 23.6 | 24.0 | 23.8 | 2.8 |
| Average | | 22.2 | 23.3 | 23.8 | 24.0 | 8.2 |

*One mouse escaped; not included in 15 day weights.

Table 4C

The Immediate Toxicity of Specific Water Samples When Given Orally to Mice (Lot M 10)

| Sample Dosage ml/kg | Deaths (10 mice/ per dose) | Average Body Weight (g) (day after dosage) 0 | 3 | 7 | 14 | Change in Weight % |
|---|---|---|---|---|---|---|
| L9 | | | | | | |
| 10 | | 24.8 | 23.2 | 24.1 | 25.6 | 3.2 |
| 25 | | 24.9 | 22.9 | 25.5 | 25.9 | 4.0 |
| 40 | | 25.0 | 23.7 | 25.4 | 26.1 | 4.4 |
| 63 | | 26.2 | 24.4 | 26.6 | 27.4 | 4.6 |
| 100 | | 25.6 | 21.7 | 23.7 | 25.2 | -1.6 |
| Average | | 25.3 | 23.2 | 25.1 | 26.0 | 2.9 |
| L10 | | | | | | |
| 10 | | 25.3 | 23.8 | 25.1 | 25.0 | -1.2 |
| 25 | | 24.5 | 23.2 | 25.2 | 25.7 | 4.9 |
| 40 | | 24.3 | 23.3 | 24.9 | 25.8 | 6.2 |
| 63 | | 25.3 | 24.1 | 26.5 | 27.0 | 6.7 |
| 100 | | 25.6 | 24.7 | 26.8 | 27.0 | 5.5 |
| Average | | 25.0 | 23.8 | 25.7 | 26.1 | 4.4 |
| L11 | | | | | | |
| 10 | | 24.6 | 23.9 | 24.8 | 25.8 | 4.9 |
| 25 | * | 25.4 | 24.5 | 26.6 | 26.8 | 5.5 |
| 40 | * | 26.2 | 25.4 | 26.3 | 27.9 | 6.6 |
| 63 | | 25.9 | 24.8 | 25.4 | 27.6 | 6.6 |
| 100 | | 23.0 | 22.1 | 24.2 | 25.0 | 8.1 |
| Average | | 25.0 | 24.1 | 25.4 | 26.6 | 6.4 |
| T9 | | | | | | |
| 10 | | 25.4 | 23.9 | 24.8 | 26.4 | 3.8 |
| 25 | | 25.8 | 24.4 | 26.6 | 26.7 | 3.4 |
| 40 | | 25.2 | 23.8 | 24.3 | 26.6 | 5.6 |
| 63 | | 26.6 | 24.4 | 26.4 | 26.8 | 0.8 |
| 100 | | 28.1 | 26.0 | 28.4 | 29.4 | 4.8 |
| Average | | 26.2 | 24.5 | 26.1 | 27.2 | 3.7 |
| T10 | | | | | | |
| 10 | | 25.6 | 25.1 | 26.4 | 27.8 | 8.9 |
| 25 | | 26.1 | 24.4 | 26.6 | 28.2 | 8.2 |
| 40 | | 23.2 | 21.7 | 23.6 | 24.2 | 4.3 |
| 63 | | 25.3 | 23.4 | 25.2 | 26.8 | 5.8 |
| 100 | | 26.1 | 24.4 | 26.4 | 26.0 | 0.4 |
| Average | | 25.3 | 23.8 | 25.6 | 26.6 | 5.3 |

*Only 9 mice per dose.

Table 4D

The Immediate Toxicity of Specific Water Samples When Given Orally to Mice (Lot M 11)

| Sample Dosage ml/kg | Deaths (10 mice/ per dose) | Average Body Weight (g) (day after dosage) 0 | 3 | 7 | 14 | Change in Weight % |
|---|---|---|---|---|---|---|
| L12 | | | | | | |
| 10 | | 30.3 | 28.6 | 30.0 | 30.7 | 1.3 |
| 25 | | 29.8 | 27.9 | 30.2 | 31.0 | 4.0 |
| 40 | | 31.2 | 27.5 | 29.4 | 31.0 | -0.6 |
| 63 | | 30.6 | 27.2 | 29.3 | 30.2 | -1.3 |
| 100 | 6 (1-3 days) | 28.6 | 22.2 | 22.9 | 26.9 | -5.9 |
| Average | | 30.1 | 27.2 | 29.1 | 30.3 | 0.8 |
| L13 | | | | | | |
| 10 | | 31.4 | 30.4 | 32.3 | 34.1 | 8.5 |
| 25 | | 28.9 | 25.8 | 28.5 | 28.2 | -2.3 |
| 40 | | 31.6 | 28.8 | 30.8 | 32.1 | 1.4 |
| 63 | | 29.8 | 26.0 | 27.6 | 29.2 | -2.2 |
| 100 | 3 (2 days) | 29.5 | 25.0 | 26.4 | 28.3 | -4.1 |
| Average | | 30.2 | 27.3 | 29.2 | 30.4 | 0.6 |
| S30 | | | | | | |
| 10 | | 28.2 | 28.1 | 30.5 | 31.3 | 10.7 |
| 25 | | 30.4 | 29.9 | 31.7 | 32.9 | 8.3 |
| 40 | | 30.2 | 29.7 | 31.1 | 32.2 | 6.3 |
| 63 | | 29.4 | 27.8 | 28.5 | 30.0 | 2.0 |
| 100 | | 29.5 | 26.7 | 28.1 | 30.3 | 2.7 |
| Average | | 29.5 | 28.4 | 30.0 | 31.3 | 6.0 |
| S31 | | | | | | |
| 10 | | 29.8 | 27.5 | 29.1 | 30.0 | 0.7 |
| 25 | | 29.3 | 28.6 | 30.7 | 31.5 | 7.3 |
| 40 | | 30.0 | 29.0 | 30.7 | 29.2 | -2.6 |
| 63 | | 30.0 | 28.3 | 30.7 | 31.7 | 5.6 |
| 100 | | 30.7 | 25.3 | 27.2 | 29.9 | -2.5 |
| Average | | 29.9 | 27.7 | 29.7 | 30.4 | 1.7 |
| S32 | | | | | | |
| 10 | | 29.0 | 27.3 | 28.3 | 28.7 | -0.9 |
| 25 | | 29.1 | 29.4 | 30.5 | 30.5 | 4.7 |
| 40 | | 28.7 | 28.0 | 29.3 | 30.3 | 5.5 |
| 63 | | 28.5 | 26.5 | 27.6 | 27.8 | -2.5* |
| 100 | | 29.1 | 26.2 | 27.9 | 88.7 | -1.3 |
| Average | | 28.9 | 27.4 | 28.7 | 29.2 | 1.1 |

*Drinking water unavailable for 18 hours.

Table 5

Summary of Results

| Water Ident. Number | Irritation Scores | | | Oral Toxicity in Mice | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Mortality | Average Wt (g) all doses (days after dose) | | | | Change |
| | Eye Sum | (N*) | Skin Aver. Score | 100 ml/kg (10 mice) | 0 | 3 | 7 | 14 | in wt. % |
| S22 | 9* | 1 | 1.6* | | 23.7 | 23.6 | 25.1 | 24.4 | 2.7 |
| S23 | 9 | | 0.6 | | 23.6 | 23.6 | 25.0 | 24.5 | 4.1 |
| S24 | 9 | | 0.4 | | 23.5 | 23.6 | 25.1 | 25.2 | 7.3 |
| S25 | 9 | | 0.3 | | 23.3 | 23.7 | 25.5 | 26.3 | 12.8 |
| S26 | 21 | 4 | 2.0 | | 23.6 | 22.9 | 24.9 | 25.6 | 8.4 |
| S27 | 8 | | 0.4 | | 23.3 | 23.3 | 24.1 | 24.9 | 7.0 |
| S28 | 9 | | 0.5 | | 22.0 | 23.4 | 23.7 | 25.1 | 14.2 |
| S29 | 9 | | 0.6 | | 23.3 | 24.1 | 24.6 | 25.0 | 4.2 |
| S30 | 5 | | 1.0 | | 29.5 | 28.4 | 30.0 | 31.3 | 6.0 |
| S31 | 6 | | 1.1 | | 29.9 | 27.7 | 29.7 | 30.4 | 1.7 |
| S32 | 11 | 2 | 1.7 | | 28.9 | 27.4 | 28.7 | 29.2 | 1.1 |
| S33 | 12 | | 1.5 | | (Incomplete) | | | | |
| T9 | 7 | | 0.6 | | 26.2 | 24.5 | 26.1 | 27.1 | 3.7 |
| T10 | 4 | | 0.4 | | 25.3 | 23.8 | 25.7 | 26.6 | 5.3 |
| L7 | 10 | | 2.6 | 1 | 22.6 | 22.9 | 23.8 | 25.2 | 11.5 |
| L8 | 2 | | 0.6 | | 22.2 | 23.3 | 23.8 | 24.0 | 8.2 |
| L9 | 12 | 2 | 3.2 | | 25.3 | 23.2 | 25.1 | 26.0 | 2.9 |
| L10 | 5 | | 0.4 | | 25.0 | 23.8 | 25.7 | 26.1 | 4.4 |
| L11 | 5 | | 0.3 | | 25.0 | 24.1 | 25.4 | 26.6 | 6.4 |
| L12 | 14 | 3 | 3.5 | 6 | 30.1 | 27.2 | 29.0 | 30.3 | 0.8 |
| L13 | 22 | 5 | 3.4 | 3 | 30.2 | 27.3 | 29.2 | 30.4 | 0.6 |

*From November Report.

Unclassified

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# THE TOXICITY AND IRRITANCY OF ULTRAFILTRATES OF NON-SANITARY MILITARY WASTES

Progress Report for January 31, 1976

Sylvan Witherup, B.S.
February 5, 1976

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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University of Cincinnati College of Medicine
Cincinnati, Ohio 45267

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## TABLE OF CONTENTS

## Introduction

This report contains data pertaining to the immediate oral toxicity and the irritant properties of ten water samples described in Table 1 and which continue in consecutive order from those described in the December Progress Report. Data pertaining to the last sample listed in the December Report, (S33) were incomplete at that time; they are completed herein.

## Results

### Skin Irritation

The average scores for erythema and for edema in the intact and abraded abdominal skin on six rabbits, immediately following and on the 3rd day after 24 hours of skin contact with the respective waters are given in Table 2. None of the samples was found to be a primary skin irritant as defined by the Procedural Regulations. The highest skin irritation scores obtained with this series of waters were 3.58, 2.48, 1.75 and 1.5 obtained with samples S-40, S-37, S-41 and S-33. The remaining six values in this series were less than one.

### Eye Irritation

The empirical scores which quantitatively summarize the irritant effects of the respective waters on the eyes of rabbits are listed in Table 3. The eye irritation consisted of no more than slight to mild palpebral erythema which was of sufficient intensity to be considered positive (a score of 2) in only one of the 60 eyes examined. None of the waters was found to be irritating to the eyes of rabbits.

Immediate Oral Toxicity

Data pertaining to the immediate toxicity of the respective waters when given orally to male mice are summarized in Tables 4A and 4B. No deaths occurred at any of the dosage levels ranging from 10 to 100 ml/kg. Two mice given waters S-37 and S-41 respectively were injured in some unknown fashion during the 5th night and were removed from their groups on the 6th morning. One mouse given water S-35 was discovered to be a female and pregnant; her weight was not included in the dose group averages. Only 9 mice were given water S-37 at each level of dosage, this sample being a concentrated water in short supply.

## Summary

All of the ten water samples tested were various concentrations of synthetic shower waste. None was found to be irritating to the eyes of rabbits.

Samples S-40 and S-37 caused moderate irritation in the skin of some rabbits, however the extensiveness of the responses was insufficient to classify the samples as primary skin irritants.

None of the waters was toxic when given orally to mice.

**Table 1**

**Identification of the Waters Studied**

| | |
|---|---|
| **S-33** | **Synthetic Shower Waste Concentrated Feed** |
| **S-34** | **Synthetic Shower Waste Normal Feed** |
| **S-35** | **Synthetic Shower Waste Concentrate Ultrafiltrate** |
| **S-36** | **Synthetic Shower Waste Feed** |
| **S-37** | **Concentrated Synthetic Shower Waste Feed** |
| **S-38** | **Synthetic Shower Waste Feed** |
| **S-39** | **Concentrated Synthetic Shower Waste Feed** |
| **S-40** | **Concentrated Synthetic Shower Waste Feed (Unfiltered)** |
| **S-41** | **Concentrated Synthetic Shower Waste Feed** |
| **S-42** | **Concentrated Real Shower Waste Ultrafiltrate** |

**Primary Irritation Induced in the Skin of Rabbits;
Scored According to Procedural Regulations**

| Condition of the Skin | Time (hrs) | Average Score (6 Rabbits) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema |
| | | S34 | | S35 | | S36 | | S37 | |
| Intact | 24 | 0.5 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 2.2 | 1.0 |
| Abraded | 24 | 1.8 | 0.8 | 1.8 | 0.2 | 2.0 | 0.8 | 2.5 | 1.8 |
| Intact | 72 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.7 | 0.0 |
| Abraded | 72 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.7 | 0.0 |
| | Sums | 2.3 | 0.8 | 1.8 | 0.2 | 3.0 | 0.8 | 7.1 | 2.8 |
| Irritation Score | | 0.78 | | 0.5 | | 0.95 | | 2.48 | |
| | | S38 | | S39 | | S40 | | S41 | |
| Intact | 24 | 0.3 | 0.0 | 1.2 | 0.0 | 2.0 | 1.7 | 1.5 | 0.7 |
| Abraded | 24 | 1.8 | 0.8 | 2.0 | 0.7 | 2.3 | 1.7 | 2.3 | 1.0 |
| Intact | 72 | 0.0 | 0.0 | 0.0 | 0.0 | 2.5 | 0.3 | 0.3 | 0.0 |
| Abraded | 72 | 0.0 | 0.0 | 0.0 | 0.0 | 3.5 | 0.3 | 1.0 | 0.2 |
| | Sums | 2.1 | 0.8 | 3.2 | 0.7 | 10.3 | 4.0 | 5.1 | 1.9 |
| Irritation Score | | 0.73 | | 0.95 | | 3.58 | | 1.75 | |

Table 2 (Continued)

Primary Irritation Induced in the Skin of Rabbits;
Scored According to Procedural Regulations

| Condition of the Skin | Time (hrs) | Average Score (6 Rabbits) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema |
| | | S-42 | | | | | | | |
| Intact | 24 | 0.7 | 0.0 | | | | | | |
| Abraded | 24 | 1.8 | 0.3 | | | | | | |
| Intact | 72 | 0.0 | 0.0 | | | | | | |
| Abraded | 72 | 0.0 | 0.0 | | | | | | |
| | Sums | 2.5 | 0.3 | | | | | | |
| Irritation Score | | 0.7 | | | | | | | |
| Intact | 24 | | | | | | | | |
| Abraded | 24 | | | | | | | | |
| Intact | 72 | | | | | | | | |
| Abraded | 72 | | | | | | | | |
| | Sums | | | | | | | | |
| Irritation Score | | | | | | | | | |

Table 3

Eye Irritation in Rabbits Resulting When the Specified Water Was Placed in Contact with the Ocular Tissues; Responses Scored According to Procedural Regulations at the Times Specified

| Water Ident. Number | Erythema* in the Conjunctiva (Sum of Scores in 6 Rabbits) Lapsed Hours 24 | 48 | 72 | Total Score | Number of Rabbits Judged Positive |
|---|---|---|---|---|---|
| S34 | 0 | 1 | 1 | 2 | 0 |
| S35 | 0 | 2 | 1 | 3 | 0 |
| S36 | 2 | 1 | 3 | 6 | 0 |
| S37 | 3 | 2 | 1 | 6 | 0 |
| S38 | 2 | 2 | 2 | 6 | 1 |
| S39 | 0 | 1 | 0 | 1 | 0 |
| S40 | 3 | 2 | 1 | 6 | 0 |
| S41 | 0 | 0 | 0 | 0 | 0 |
| S42 | 2 | 2 | 2 | 6 | 0 |

*There was no chemosis in the conjunctiva, no ulceration or clouding of the cornea and no effect on the iris resulting at any time from contact with the specified waters.

## Table 4A

## The Immediate Toxicity of Specific Water Samples When Given Orally to Mice (Lot M12)

| Sample Dosage ml/kg | Deaths (10 mice/ per dose) | Average Body Weight (g) (day after dosage) 0 | 3 | 7 | 14 | Change in Weight % |
|---|---|---|---|---|---|---|
| S33 | | | | | | |
| 10 | | 30.0 | 29.0 | 30.4 | 30.8 | 2.6 |
| 25 | | 31.8 | 30.0 | 30.4 | 31.5 | -0.9 |
| 40 | | 31.3 | 27.5 | 29.4 | 30.7 | -1.8 |
| 63 | | 32.0 | 29.6 | 30.3 | 32.2 | 0.4 |
| 100 | | 31.1 | 29.1 | 30.1 | 32.1 | 3.2 |
| Average | | 31.2 | 29.0 | 30.1 | 31.4 | 0.7 |
| S34 | | | | | | |
| 10 | | 30.0 | 31.0 | 32.4 | 32.9 | 9.6 |
| 25 | | 31.5 | 30.3 | 30.5 | 32.2 | 2.1 |
| 40 | | 31.8 | 31.8 | 29.6 | 32.2 | 1.1 |
| 63 | | 31.7 | 28.6 | 31.0 | 32.3 | 1.8 |
| 100 | | 31.7 | 27.7 | 30.3 | 31.8 | 0.2 |
| Average | | 31.4 | 29.8 | 30.8 | 32.3 | 2.9 |
| S35 | | | | | | |
| 10 | | 30.5 | 30.4 | 31.3 | 32.2 | 5.3 |
| 25 | | 33.5 | 33.2 | 32.9 | 34.1 | 1.6 |
| 40 | | 31.6 | 30.5 | 30.2 | 31.0 | -2.0 |
| 63 | * | 32.1 | 32.1 | 31.0 | 33.1 | 3.1 |
| 100 | | 32.1 | 30.4 | 31.3 | 32.1 | -0.1 |
| Average | | 32.0 | 31.3 | 31.3 | 32.5 | 1.5 |
| S36 | | | | | | |
| 10 | | 31.6 | 32.4 | 33.0 | 34.0 | 7.6 |
| 25 | | 30.6 | 29.7 | 29.8 | 30.7 | 0.4 |
| 40 | | 31.7 | 31.4 | 32.1 | 32.5 | 2.3 |
| 63 | | 32.0 | 32.0 | 32.4 | 33.5 | 4.4 |
| 100 | | 31.7 | 31.2 | 31.8 | 32.6 | 2.8 |
| Average | | 31.5 | 31.3 | 31.8 | 32.6 | 3.5 |
| S37 | | | | | | |
| 10 | | 31.3 | 32.0 | 33.5 | 32.9 | 4.8 |
| 25 | 0/9 | 34.2 | 33.6 | 33.9 | 35.4 | 3.3 |
| 40 | 0/9 | 34.5 | 34.8 | 34.6 | 35.6 | 3.2 |
| 63 | 1/9(K-6d) | 34.4 | 33.7 | 33.3 | 34.2 | -0.6 |
| 100 | 0/8 | 35.1 | 33.4 | 34.1 | 35.0 | -0.3 |
| Average | | 33.8 | 33.2 | 33.9 | 34.6 | 2.2 |

*pregnant female removed before 7th day weighing
**killed because of physical injury.

Table 4B

The Immediate Toxicity of Specific Water Samples When Given Orally to Mice (Lot M13)

| Sample Dosage ml/kg | Deaths (10 mice/ per dose) | Average Body Weight (g) (day after dosage) 0 | 3 | 7 | 14 | Change In Weight % |
|---|---|---|---|---|---|---|
| S38 | | | | | | |
| 10 | | 32.6 | 32.3 | 32.8 | 33.5 | 2.6 |
| 25 | | 33.9 | 33.5 | 34.4 | 35.0 | 3.1 |
| 40 | | 33.7 | 34.0 | 34.4 | 35.6 | 5.6 |
| 63 | | 33.6 | 33.5 | 33.6 | 34.0 | 1.3 |
| 100 | | 33.5 | 32.7 | 32.9 | 33.9 | 1.2 |
| Average | | 33.5 | 33.2 | 33.6 | 34.4 | 2.8 |
| S39 | | | | | | |
| 10 | | 31.7 | 31.2 | 32.0 | 32.2 | 1.5 |
| 25 | | 33.4 | 33.6 | 33.9 | 34.6 | 3.5 |
| 40 | | 33.7 | 33.4 | 33.3 | 34.6 | 2.4 |
| 63 | | 33.7 | 33.4 | 33.6 | 34.4 | 1.8 |
| 100 | | 33.6 | 33.5 | 33.5 | 34.5 | 2.6 |
| Average | | 33.2 | 33.0 | 33.2 | 34.0 | 2.4 |
| S40 | | | | | | |
| 10 | | 32.7 | 31.6 | 32.4 | 32.6 | -0.4 |
| 25 | | 33.7 | 32.5 | 32.9 | 34.1 | 1.2 |
| 40 | | 32.5 | 32.1 | 32.4 | 33.8 | 4.0 |
| 63 | | 32.3 | 31.2 | 31.7 | 33.3 | 3.0 |
| 100 | | 32.3 | 29.6 | 30.1 | 31.5 | -2.6 |
| Average | | 32.7 | 31.4 | 31.9 | 33.0 | 1.0 |
| S41 | | | | | | |
| 10 | | 32.5 | 32.3 | 33.3 | 33.2 | 2.1 |
| 25 | | 33.2 | 33.1 | 33.3 | 35.0 | 5.4 |
| 40 | | 33.4 | 33.3 | 33.3 | 34.8 | 4.1 |
| 63 | | 32.6 | 33.0 | 33.2 | 34.4 | 5.3 |
| 100 | 1 (k-6 days) | 33.8 | 31.6 | 33.3 | 34.4 | 1.9 |
| Average | | 33.1 | 32.6 | 33.3 | 34.3 | 3.7 |
| S42 | | | | | | |
| 10 | | 33.3 | 33.0 | 33.9 | 34.9 | 4.7 |
| 25 | | 32.5 | 31.8 | 32.7 | 34.0 | 4.3 |
| 40 | | 33.9 | 34.2 | 34.6 | 35.1 | 3.7 |
| 63 | | 32.7 | 32.1 | 32.0 | 33.7 | 3.0 |
| 100 | | 31.2 | 31.0 | 31.2 | 32.4 | 4.0 |
| Average | | 32.7 | 32.4 | 32.9 | 34.0 | 3.9 |

(k) killed because of physical injury.

Table 5

Summary of Results

| Water Ident. Number | Irritation Scores | | | Oral Toxicity in Mice | | | | | Change in wt. % |
|---|---|---|---|---|---|---|---|---|---|
| | Eye Sum | (N*) | Skin Aver. Score | Mortality 100 ml/kg (10 mice) | Average Wt (g) all doses (days after dose) 0 | 3 | 7 | 14 | |
| S33** | 12 | | 1.5 | | 31.2 | 29.0 | 30.1 | 31.4 | 0.7 |
| S34 | 2 | | 0.78 | | 31.4 | 29.8 | 30.8 | 32.3 | 2.9 |
| S35 | 3 | | 0.5 | | 32.0 | 31.3 | 31.3 | 32.5 | 1.5 |
| S36 | 6 | | 0.95 | | 31.5 | 31.3 | 31.8 | 32.6 | 3.5 |
| S37 | 6 | | 2.48 | | 33.8 | 33.2 | 33.9 | 34.6 | 2.2 |
| S38 | 6 | 1 | 0.73 | | 33.5 | 33.2 | 33.6 | 34.4 | 2.8 |
| S39 | 1 | | 0.95 | | 33.2 | 33.0 | 33.2 | 34.0 | 2.4 |
| S40 | 6 | | 3.58 | | 32.7 | 31.4 | 31.9 | 33.0 | 1.0 |
| S41 | 0 | | 1.75 | 1 (k) | 33.1 | 32.6 | 33.3 | 34.3 | 3.7 |
| S42 | 6 | | 0.7 | | 32.7 | 32.4 | 32.9 | 34.0 | 3.9 |

*Number of rabbits judged positive

**Irritation scores for rabbits previously reported, December 31, 1975.

(k) One mouse killed on day 6 because of physical injury.

Unclassified

AD _____

# THE TOXICITY AND IRRITANCY OF ULTRAFILTRATES OF NON-SANITARY MILITARY WASTES

Progress Report for Feb. 1 - Mar. 31, 1976

Sylvan Witherup, B.S.

April 8, 1976

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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University of Cincinnati College of Medicine
Cincinnati, Ohio 45267

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## TABLE OF CONTENTS

## INTRODUCTION

This report contains data pertaining to the immediate oral toxicity and the irritant properties of 14 water samples described in Table 1 and which continue in consecutive order from those described in the January Progress Report (Feb. 5, 1976).

## RESULTS

### Skin Irritation

The average scores for erythema and edema in the intact and abraded abdominal skin on six rabbits, immediately following 24 hours of contact with the respective waters and 48 hours after the contact was terminated are given in Table 2. Of the 14 waters tested, Sample S-43, a concentrated synthetic shower waste feed water, with an average score of 3.0 was the most irritating to the skin. Sample S-44 was 2nd high with a score of 2.1; Sample S-45 with a score of 1.65 was 3rd in order of severity. The remaining 11 samples evoked scores ranging from 0.6 to 1.63.

### Eye Irritation

The empirical scores which quantitatively summarize the irritant effects of the respective waters on the eyes of rabbits are listed in Table 3. The eye irritation consisted of no more than mild to moderate palpebral erythema which was of sufficient intensity to be considered positive (a score of 2 or more) in 4 of the 84 eyes examined; one of six rabbits showed a positive response in each of the four groups subjected respectively to contact with waters S-43, S-44, S-45, and S-46. In no instance was there any edema in the palpebrum or involvement of the cornea or iris.

## Immediate Oral Toxicity

Data pertaining to the immediate toxicity of the respective waters when given orally to mice are summarized in Tables 4A, 4B and 4C. One of ten mice in each of 4 groups given respectively 100 ml/kg of L-14, S-45, S-47 and S-51 died in 1 to 8 days. One of ten mice given 63 ml/kg of S-43 died after 8 days; there was also one death in each of three groups given 25 ml/kg of S-51, S-52 and S-55 but these deaths may have been incidental since in each instance 29 mice survived much larger dosages of the same water.

Mice given large dosages (40 to 100 ml/kg) of the concentrated waters developed tremors,mild ataxia, excessive urination, diarrhea, anorexia, inanition and weight loss, with recovery in 7 to 14 days. Body weights of the mice may have been influenced to some extent by malfunctioning of the room temperature control system which permitted the room temperature to drop to 50-60°F on the 11th and 13th days. Exceptional weight gains of the mice in Lot M-16 given samples S52, 53, 54 and 55) were in large part attributable to the fact that these animals were younger than those in lots M-14 and M-15.

## SUMMARY

The quantitative data pertaining to the effects of the respective waters when kept for 24 hours in contact with the intact and abraded abdominal skin of rabbits, when placed in contact with the ocular tissues of rabbits and when given orally to mice are summarized in Table 5. Physiological changes of sufficient severity to warrent concern occurred only with the highly concentrated samples.

Table 1

Identification of the Waters Studied

L14- Concentrated US Army Detergent, Filtered

S43- Concentrated Synthetic Shower Waste Feed, Filtered

S44- Concentrated Synthetic Shower Waste Feed, Filtered

S45- Concentrated Synthetic Shower Waste Feed, Filtered

S46- Concentrated Synthetic Shower Waste Feed, Filtered

S47- Concentrated Synthetic Shower Waste Feed, Filtered

S48- Concentrated Real Shower Waste Feed, Filtered

S49- Real Shower Waste Ultrafiltrate

S50- Concentrated Actual Shower Waste Feed

S51- Filtered, Concentrated Actual Shower Waste Feed

S52- Filtered, Actual Shower Waste Feed

S53- Actual Shower Waste Ultrafiltrate

S54- Filtered Actual Shower Waste Feed

S55- Actual Shower Waste Ultrafiltrate

Table 2

Primary Irritation Induced in the Skin of Rabbits;
Scored According to Procedural Regulations

| Condition of the Skin | Time (hrs) | Average Score (6 Rabbits) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema |
| | | L14 | | S43 | | S44 | | S45 | |
| Intact | 24 | 1.0 | 0.7 | 1.5 | 0.5 | 1.8 | 0.8 | 1.2 | 0.3 |
| Abraded | 24 | 1.8 | 1.2 | 2.7 | 1.8 | 1.8 | 1.2 | 2.5 | 1.0 |
| Intact | 72 | 0.2 | 0.0 | 2.0 | 0.2 | 0.8 | 0.7 | 0.3 | 0.0 |
| Abraded | 72 | 0.0 | 0.0 | 2.3 | 1.0 | 0.8 | 0.5 | 1.0 | 0.3 |
| | Sums | 3.0 | 1.9 | 8.5 | 3.5 | 5.2 | 3.2 | 5.0 | 1.6 |
| Irritation Score | | 1.23 | | 3.0 | | 2.1 | | 1.65 | |
| | | S46 | | S47 | | S48 | | S49 | |
| Intact | 24 | 0.7 | 0.3 | 1.2 | 0.8 | 1.3 | 0.5 | 1.2 | 0.0 |
| Abraded | 24 | 2.0 | 0.7 | 1.7 | 1.3 | 2.0 | 1.2 | 1.8 | 1.5 |
| Intact | 72 | 0.0 | 0.0 | 0.3 | 0.0 | 0.0 | 0.0 | 0.2 | 0.0 |
| Abraded | 72 | 0.2 | 0.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 0.0 |
| | Sums | 2.9 | 1.3 | 3.2 | 2.1 | 3.3 | 1.7 | 3.4 | 1.5 |
| Irritation Score | | 1.05 | | 1.33 | | 1.25 | | 1.23 | |

Table 2 (Continued)

Primary Irritation Induced in the Skin of Rabbits;
Scored According to Procedural Regulations

| Condition of the Skin | Time (hrs) | Average Score (6 Rabbits) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema |
| | | S50 | | S51 | | S52 | | S53 | |
| Intact | 24 | 0.0 | 0.0 | 0.2 | 0.3 | 0.7 | 0.2 | 0.3 | 0.2 |
| Abraded | 24 | 0.7 | 0.8 | 0.7 | 1.3 | 1.7 | 1.2 | 1.8 | 1.0 |
| Intact | 72 | 0.2 | 0.0 | 0.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Abraded | 72 | 0.7 | 0.0 | 1.0 | 0.0 | 0.2 | 0.0 | 0.0 | 0.0 |
| | Sums | 1.6 | 0.8 | 2.4 | 1.6 | 2.6 | 1.4 | 2.1 | 1.2 |
| Irritation Score | | 0.6 | | 1.0 | | 1.0 | | 0.83 | |
| | | S54 | | S55 | | | | | |
| Intact | 24 | 0.5 | 0.2 | 0.5 | 0.2 | | | | |
| Abraded | 24 | 2.2 | 1.8 | 2.0 | 2.0 | | | | |
| Intact | 72 | 0.0 | 0.0 | 0.0 | 0.0 | | | | |
| Abraded | 72 | 1.0 | 0.5 | 1.3 | 0.5 | | | | |
| | Sums | 3.7 | 2.5 | 3.8 | 2.7 | | | | |
| Irritation Score | | 1.55 | | 1.63 | | | | | |

Table 3

Eye Irritation in Rabbits Resulting When the Specified Water Was Placed in Contact with the Ocular Tissues; Responses Scored According to Procedural Regulations at the Times Specified

| Water Ident. Number | Erythema* in the Conjunctiva (Sum of Scores in 6 Rabbits) Lapsed Hours 24 R** | 48 R** | 72 R** | Total Score | Number of Rabbits Judged Positive |
|---|---|---|---|---|---|
| L14 | 2 | 3 | 2 | 7 | 0 |
| S43 | 7 | 1 | 4 | 12 | 1 |
| S44 | 5 | 2 | 4 | 11 | 1 |
| S45 | 5 | 5 | 6 | 16 | 1 |
| S46 | 6 | 5 | 5 | 16 | 1 |
| S47 | 4 | 2 | 1 | 7 | 0 |
| S48 | 3 | 2 | 2 | 7 | 0 |
| S49 | 4 | 2 | 2 | 8 | 0 |
| S50 | 1 | 2 | 2 | 5 | 0 |
| S51 | 5 | 2 | 1 | 8 | 0 |
| S52 | 3 | 3 | 2 | 8 | 0 |
| S53 | 2 | 4 | 1 | 6 | 0 |
| S54 | 4 | 3 | 5 | 12 | 0 |
| S55 | 4 | 3 | 4 | 11 | 0 |

*There was no swelling of the palpebrum, no clouding or ulceration of the the cornea and no noticeable change in the iris. Pupillary responses to light were normal.

**R = redness

Table 4A

The Immediate Toxicity of Specific Water Samples When Given Orally to Mice (Lot M14)

| Sample | Dosage ml/kg | Deaths (10 mice/ per dose) | Average Body Weight (g) (day after dosage) 0 | 3 | 7 | 14 | Change In Weight % |
|---|---|---|---|---|---|---|---|
| S43 | 10 | | 32.4 | 32.0 | 31.8 | 32.6 | 0.46 |
| | 25 | | 33.6 | 33.4 | 33.4 | 35.0 | 4.17 |
| | 40 | | 33.6 | 32.8 | 31.9 | 32.3 | -4.13 |
| | 63 | 1(8 days) | 32.1 | 31.9 | 32.7 | 33.4 | 4.17 |
| | 100 | | 33.1 | 33.1 | 33.4 | 34.1 | 3.02 |
| Average | | | 33.0 | 32.6 | 32.6 | 33.5 | 1.52 |
| S44 | 10 | | 32.4 | 32.1 | 32.0 | 32.9 | 1.39 |
| | 25 | | 33.3 | 33.2 | 33.6 | 35.1 | 5.53 |
| | 40 | | 32.1 | 31.4 | 31.7 | 30.8 | -3.93 |
| | 63 | | 33.2 | 32.6 | 33.0 | 33.5 | 0.78 |
| | 100 | | 34.0 | 33.8 | 33.7 | 34.1 | 0.21 |
| Average | | | 33.0 | 32.6 | 32.8 | 33.3 | 0.82 |
| S45 | 10 | | 32.7 | 32.3 | 32.0 | 32.6 | -0.37 |
| | 25 | | 31.3 | 31.5 | 32.1 | 32.9 | 4.95 |
| | 40 | | 32.4 | 32.7 | 34.2 | 34.2 | 5.53 |
| | 63 | | 33.4 | 32.8 | 33.6 | 34.9 | 4.40 |
| | 100 | 1(8 days) | 33.2 | 32.6 | 31.9 | 32.3 | -2.66 |
| Average | | | 32.6 | 32.4 | 32.8 | 33.4 | 2.40 |
| S46 | 10 | | 33.1 | 32.9 | 33.0 | 34.9 | 5.19 |
| | 25 | | 31.6 | 32.1 | 32.9 | 34.8 | 9.83 |
| | 40 | | 32.0 | 32.2 | 32.6 | 34.2 | 6.75 |
| | 63 | | 32.7 | 32.9 | 33.7 | 34.5 | 5.33 |
| | 100 | | 31.2 | 30.4 | 31.5 | 32.3 | 3.56 |
| Average | | | 32.1 | 32.1 | 32.7 | 34.1 | 6.10 |
| S47 | 10 | | 32.6 | 32.3 | 33.4 | 34.5 | 5.74 |
| | 25 | | 32.6 | 32.9 | 33.8 | 35.0 | 7.27 |
| | 40 | | 33.6 | 34.1 | 34.9 | 36.6 | 8.38 |
| | 63 | | 33.2 | 33.2 | 33.3 | 34.9 | 5.18 |
| | 100 | 1(1 day) | 32.7 | 30.9 | 32.1 | 32.5 | -0.49 |
| Average | | | 33.0 | 32.7 | 33.5 | 34.7 | 5.37 |

Table 4B

The Immediate Toxicity of Specific Water Samples When Given Orally to Mice (Lot M15)

| Sample | Dosage ml/kg | Deaths (10 mice/ per dose) | Average Body Weight (g) (day after dosage) 0 | 3 | 7 | 14 | Change In Weight % |
|---|---|---|---|---|---|---|---|
| L14 | 10 | | 33.3 | 32.8 | 33.8 | 30.8 | -7.63 |
| | 25 | | 34.1 | 32.7 | 33.2 | 35.3 | 3.67 |
| | 40 | | 35.2 | 34.7 | 35.4 | 36.0 | 2.30 |
| | 63 | | 32.9 | 31.6 | 32.5 | 33.2 | 0.91 |
| | 100 | 1(2 days) | 36.1 | 34.2 | 36.3 | 37.2 | 3.25 |
| Average | | | 34.3 | 33.1 | 34.4 | 34.4 | 0.42 |
| S48 | 10 | | 32.7 | 32.3 | 32.7 | 33.4 | 2.14 |
| | 25 | | 35.2 | 34.1 | 35.4 | 36.5 | 3.72 |
| | 40 | | 33.3 | 31.9 | 32.9 | 33.7 | 1.23 |
| | 63 | | 35.1 | 33.6 | 35.4 | 36.0 | 2.68 |
| | 100 | | 33.5 | 32.3 | 33.3 | 34.6 | 3.19 |
| Average | | | 33.9 | 32.8 | 33.9 | 35.5 | 2.61 |
| S49 | 10 | | 31.7 | 31.2 | 32.0 | 33.6 | 6.16 |
| | 25 | | 32.8 | 32.1 | 32.9 | 34.5 | 5.09 |
| | 40 | | 34.6 | 32.6 | 34.2 | 34.6 | 0.14 |
| | 63 | | 33.0 | 31.4 | 32.8 | 34.0 | 3.06 |
| | 100 | | 34.3 | 33.0 | 33.8 | 34.9 | 1.81 |
| Average | | | 33.3 | 32.0 | 33.1 | 34.3 | 3.19 |
| S50 | 10 | | 33.2 | 31.9 | 32.7 | 30.3 | -8.91 |
| | 25 | | 34.2 | 33.4 | 34.7 | 35.1 | 2.78 |
| | 40 | | 31.9 | 31.1 | 32.7 | 33.0 | 3.51 |
| | 63 | | 33.7 | 32.4 | 33.7 | 33.6 | -0.27 |
| | 100 | | 34.4 | 32.9 | 34.7 | 33.9 | -1.57 |
| Average | | | 33.5 | 32.3 | 33.7 | 33.2 | -0.91 |
| S51 | 10 | | 34.0 | 33.2 | 33.9 | 33.7 | -0.94 |
| | 25 | 1 (6 days)* | 32.5 | 30.3 | 32.1 | 32.5 | 0.01 |
| | 40 | | 32.7 | 32.3 | 33.5 | 33.5 | 2.48 |
| | 63 | | 33.0 | 32.5 | 33.5 | 34.1 | 3.46 |
| | 100 | 1 (3 days) | 32.6 | 32.8 | 33.9 | 34.7 | 6.35 |
| Average | | | 33.0 | 32.2 | 33.4 | 33.7 | 2.29 |

*Only 9 mice in this dose group.

Table 4C

The Immediate Toxicity of Specific Water Samples When Given Orally to Mice (Lot M16 )

| Sample | Dosage ml/kg | Deaths (10 mice/ per dose) | Average Body Weight (g) (day after dosage) 0 | 3 | 7 | 14 | Change In Weight % |
|---|---|---|---|---|---|---|---|
| S52 | 10 | | 25.4 | 26.0 | 27.8 | 30.5 | 20.0 |
| | 25 | 1(7 days) | 25.6 | 24.8 | 27.7 | 30.5 | 19.0 |
| | 40 | | 27.7 | 27.2 | 29.2 | 33.6 | 21.5 |
| | 63 | | 26.0 | 26.1 | 28.4 | 31.7 | 22.1 |
| | 100 | | 24.8 | 24.8 | 27.5 | 31.9 | 16.8 |
| Average | | | 26.3 | 25.8 | 28.1 | 31.6 | 20.0 |
| S53 | 10 | | 27.6 | 26.6 | 28.6 | 31.6 | 14.3 |
| | 25 | | 27.5 | 26.9 | 29.4 | 33.0 | 19.9 |
| | 40 | | 26.2 | 27.1 | 29.2 | 33.1 | 26.3 |
| | 63 | | 26.7 | 26.6 | 29.1 | 32.6 | 22.0 |
| | 100 | | 26.3 | 26.1 | 28.5 | 32.1 | 21.6 |
| Average | | | 26.9 | 26.6 | 29.0 | 32.5 | 20.7 |
| S54 | 10 | | 25.5 | 25.8 | 27.6 | 30.4 | 19.0 |
| | 25 | | 26.1 | 26.5 | 28.8 | 32.3 | 23.7 |
| | 40 | | 27.0 | 27.4 | 29.8 | 33.0 | 21.9 |
| | 63 | | 26.4 | 27.4 | 30.0 | 33.2 | 25.4 |
| | 100 | | 26.7 | 27.0 | 29.6 | 32.7 | 22.1 |
| Average | | | 26.3 | 26.8 | 29.1 | 32.3 | 22.4 |
| S55 | 10 | 1 (14 days) | 25.9 | 25.8 | 27.2 | 29.7 | 14.5 |
| | 25 | 1 (1 day) | 26.9 | 26.7 | 28.8 | 32.4 | 20.6 |
| | 40 | | 25.9 | 25.9 | 28.1 | 31.4 | 21.1 |
| | 63 | | 26.1 | 27.3 | 29.8 | 33.0 | 26.3 |
| | 100 | | 28.0 | 26.2 | 28.9 | 32.3 | 15.5 |
| Average | | | 26.6 | 26.4 | 28.5 | 31.8 | 19.7 |

Table 5

Summary of Results

| Water Ident. Number | Irritation Scores | | Oral Toxicity in Mice | | | | | Change in Wt. % |
|---|---|---|---|---|---|---|---|---|
| | Eye Sum (N*) | Skin Aver. Score | Mortality 100 ml/kg (10 mice) | Average Wt (g) all doses (days after dose) 0 | 3 | 7 | 14 | |
| L14 | 7 - 0 | 1.23 | 1 (2 days) | 34.3 | 33.1 | 34.4 | 34.4 | 0.42 |
| S43 | 12 - 1 | 3.0 | | 33.0 | 32.6 | 32.6 | 33.5 | 1.52 |
| S44 | 11 - 1 | 2.1 | | 33.0 | 32.6 | 32.8 | 33.3 | 0.82 |
| S45 | 16 - 1 | 1.65 | 1 (8 days) | 32.6 | 32.4 | 32.8 | 33.4 | 2.40 |
| S46 | 16 - 1 | 1.05 | | 32.1 | 32.1 | 32.7 | 34.1 | 6.10 |
| S47 | 7 - 0 | 1.33 | 1 (1 day) | 33.0 | 32.7 | 33.5 | 34.7 | 5.37 |
| S48 | 7 - 0 | 1.25 | | 33.9 | 32.8 | 33.9 | 35.5 | 2.61 |
| S49 | 8 - 0 | 1.23 | | 33.3 | 32.0 | 33.1 | 34.3 | 3.19 |
| S50 | 5 - 0 | 0.6 | | 33.5 | 32.3 | 33.7 | 33.2 | -0.9i |
| S51 | 8 - 0 | 1.0 | 1 (3 days) | 33.0 | 32.2 | 33.4 | 33.7 | 2.29 |
| S52 | 7 - 0 | 1.0 | | 26.3 | 25.8 | 28.1 | 31.6 | 20.0 |
| S53 | 8 - 0 | 0.83 | | 26.9 | 26.6 | 29.0 | 32.5 | 20.7 |
| S54 | 12 - 0 | 1.55 | | 26.3 | 26.8 | 29.1 | 32.3 | 22.4 |
| S55 | 11 - 0 | 1.63 | | 26.6 | 26.4 | 28.5 | 31.8 | 19.7 |

*Number judged positive.

Unclassified

AD _____

# THE TOXICITY AND IRRITANCY OF ULTRAFILTRATES OF NON-SANITARY MILITARY WASTES

Progress Report for
April 1, - May 31, 1976

Sylvan Witherup, B.S.
June 8, 1976

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Washington, D.C. 20314

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University of Cincinnati College of Medicine
Cincinnati, Ohio 45267

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## Introduction

This report contains data pertaining to the immediate oral toxicity and the irritant properties of the fourteen water samples described in Table 1, and which continue in consecutive order from those described in the Progress Report for February 1 - March 31, 1976 (April 8, 1976).

## Results

### Skin Irritation

Table 2 lists the average scores for erythema and for edema in the intact and abraded abdominal skin of six rabbits, immediately following 24 hours of contact with the respective waters, and 48 hours after the contact was terminated. Nine of the samples, S56 - 59 and L17 - 21, were judged to be corrosive substances, causing necrosis (and also, in some animals ulceration and eschar formation in the skin). Because corrosion is more severe than the effects scored by procedural regulations, average irritation scores for these samples are not given.

Sample L15, with an average score of 5.40, was found to be a primary irritant, causing ulceration and eschar formation. Testing with sample L16 was limited to one rabbit because of a technical error related to an insufficient volume of the water. The remaining three samples, S60, 61 and 62, had average scores of 0.98, 1.13, and 1.15, respectively.

Eye Irritation

A summary of the eye irritation scores is provided in Table 3. The severity and extent of the responses was sufficient to classify samples S56, S58, L15, L17, and L21 as eye irritants. Irritation responses to these waters consisted of severe erythema and moderate chemosis of the palpebral conjunctiva. Positive responses in the cornea and in the iris were also noted. The laundry samples induced generally more severe reactions than did the shower samples.

The responses to 557 and L18 are equivocal, since there was a positive response in 3 of the 6 rabbits treated in each test. An additional test is needed to classify each water; however, in consideration of the skin irritation and the oral toxicity resulting from these samples (Tables 2 and 4, respectively), both waters should be considered eye irritants until more tests are performed.

Testing with sample L16 could not be completed. Eye irritations caused by the remaining samples (S59-62, L19-20) consisted of no more than mild palpebral erythema. There was no chemosis in the conjunctivae nor involvement of the cornea or iris.

Immediate Oral Toxicity

Data pertaining to the immediate toxicity of the respective waters when given orally to mice are summarized in Tables 4A, B, and C. The LD50's of some specific waters are shown in Figure 1.

Except for samples S59-62, all animals treated at 100ml/kg and a high percentage of the mice treated at 63 and 40 ml/kg died. Only 4

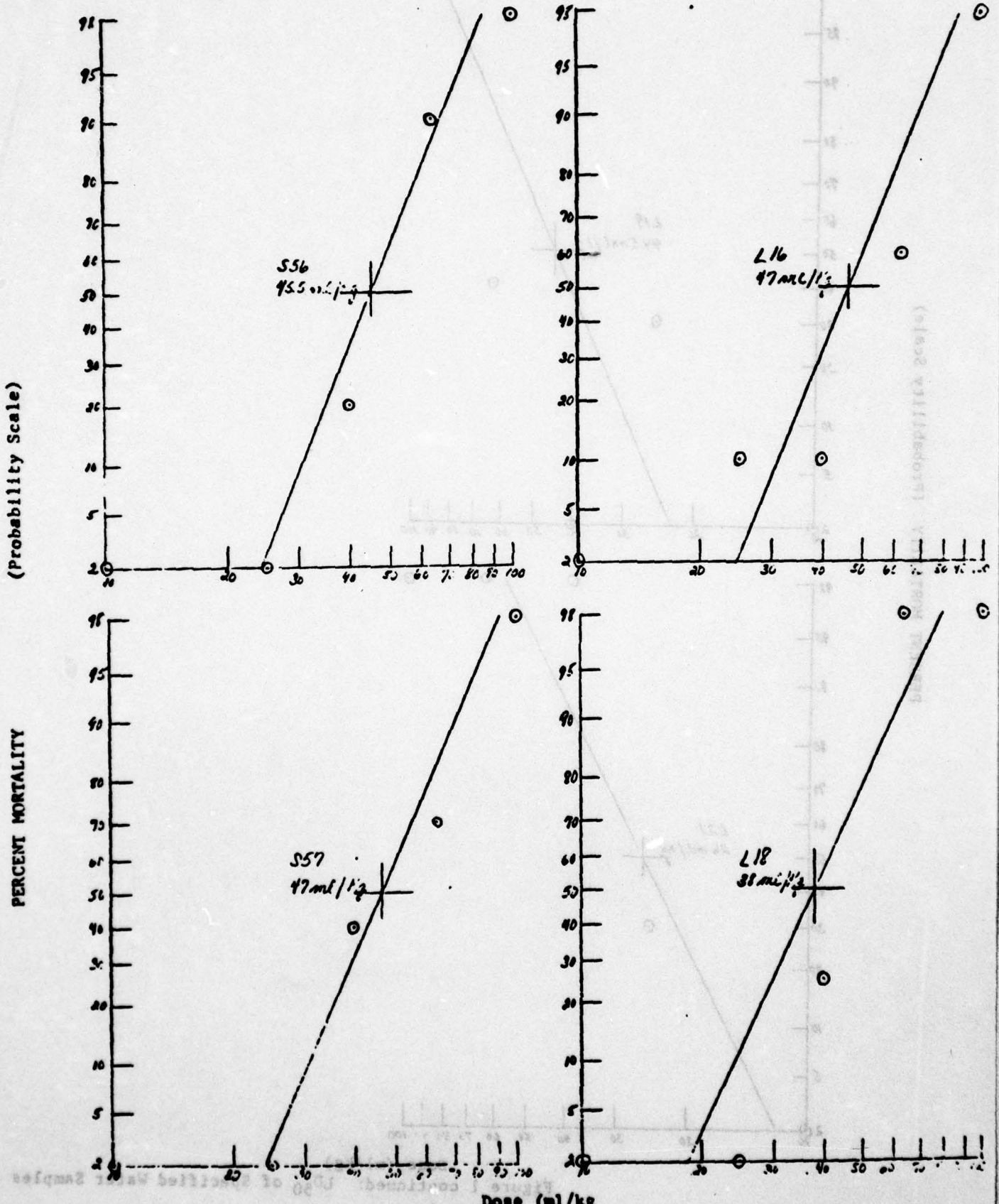


Figure 1: $LD_{50}$ of Specified Water Samples

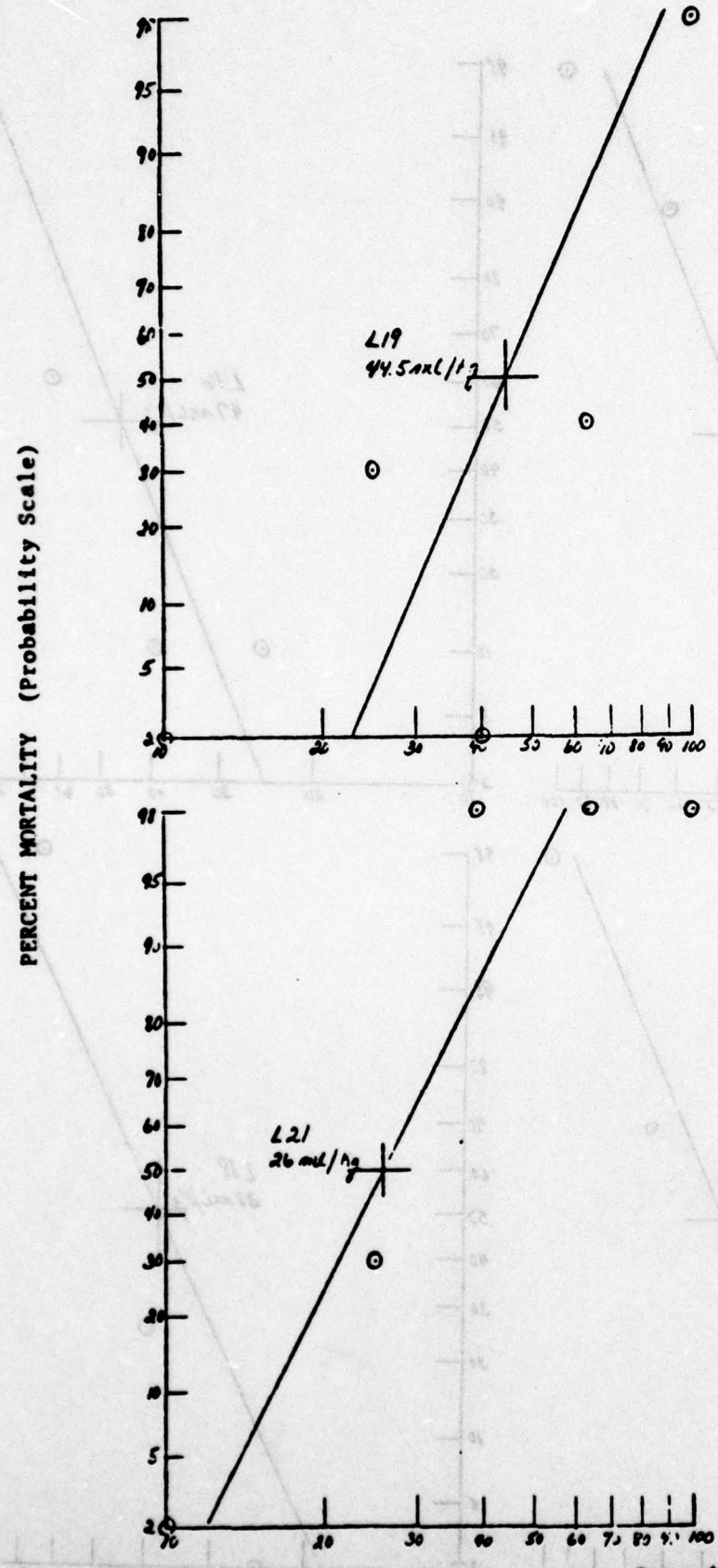


Figure 1 continued: $LD_{50}$ of Specified Water Samples

samples (L16, 19, 20, and 21) caused deaths at 25 ml/kg. There were no deaths at 10 ml/kg of any water. Twenty-seven per cent of the deaths occurred in less than 1 hour, 55% in less than 12 hours, 93% in less than 24 hours, 100% in less than 3 days after treatment. In general, the signs of illness before death included ataxia, labored breathing, tremors, and convulsions.

Sample S59 caused 2 deaths of 9 mice treated at 100 ml/kg. There was no mortality among animals treated with S60. Most animals that survived, in all 14 tests, experienced some weight loss, but generally recovered in 7-14 days.

Some of the waters were in short supply, so 10 animals were not treated at all dose levels.

## Summary

Fourteen water samples have been tested with respect to their irritancy and oral toxicity. The observations are summarized in Table 5. Samples S60, 61, and 62 produced no eye or skin irritations on rabbits and were not toxic to mice. Sample S59 was corrosive to the skin of rabbits but caused no eye irritation or oral toxicity. The other 10 samples caused severe physiological changes in rabbits and a high incidence of mortality in mice; these eleven samples were highly concentrated.

TABLE 1

**Identification of the Waters Studied**

S56 - Unfiltered Concentrated Synthetic Shower Waste

S57 - Unfiltered Concentrated Synthetic Shower Waste

S58 - Concentrated Synthetic Shower Waste Filtrate

S59 - Concentrated Synthetic Shower Waste Filtered

S60 - Multiple Pass Concentrate from Real Shower Waste

S61 - Unfiltered Real Shower Waste

S62 - Filtered Real Shower Waste

L15 - Concentrated Laundry Waste, Filtered

L16 - Concentrated Laundry Waste, Filtered

L17 - Concentrated Mixture of Synthetic Shower and Laundry Waste, Filtered

L18 - Concentrated Army Detergent

L19 - Mixture Concentrated Army Detergent and Concentrated Synthetic Shower Waste

L20 - Concentrated Army Detergent

L21 - Unfiltered Concentrated Laundry Waste

TABLE 2

Primary Irritation Induced in the Skin of Rabbits;
Scored According to Procedural Regulations

| Condition of the Skin | Time (hrs.) | Average Score (6 Rabbits) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Erythema | Edema | Erythema | Edema | Erythema | Edema | Erythema | Edema |
| | | S56 | | S57 | | S58 | | S59 | |
| Intact | 24 | Corrosive* | | Corrosive* | | Corrosive* | | Corrosive* | |
| Abraded | 24 | | | | | | | | |
| Intact | 72 | | | | | | | | |
| Abraded | 72 | | | | | | | | |
| | Sums | | | | | | | | |
| Irritation Score | | | | | | | | | |
| | | S60 | | S61 | | S62 | | L15 | |
| Intact | 24 | 0.2 | 0.0 | 0.3 | 0.0 | 0.5 | 0.3 | 2.2 | 2.8 |
| Abraded | 24 | 2.0 | 1.5 | 2.0 | 2.0 | 2.0 | 1.0 | 4.0 | 4.0 |
| Intact | 72 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.0 | 0.8 |
| Abraded | 72 | 0.2 | 0.0 | 0.2 | 0.0 | 0.8 | 0.0 | 4.0 | 0.8 |
| | Sums | 2.4 | 1.5 | 2.5 | 2.0 | 3.3 | 1.3 | 13.2 | 8.4 |
| Irritation Score | | 0.98 | | 1.13 | | 1.15 | | 5.40 | |

*Irritation scores are not applicable to corrosive substances, since corrosion is more severe than the highest scored irritations.

TABLE 2, continued

Primary Irritation Induced in the Skin of Rabbits;
Scored According to Procedural Regulations

| Condition of the Skin | Time (hrs.) | Average Score (6 Rabbits) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Erythema | Edema | Erythema | Edema | Erythema | Edema | Erythema | Edema |
| | | L16 | | L17 | | L18 | | L19 | |
| Intact | 24 | Insufficient volume of sample to conclude the test | | Corrosive* | | Corrosive* | | Corrosive* | |
| Abraded | 24 | | | | | | | | |
| Intact | 72 | | | | | | | | |
| Abraded | 72 | | | | | | | | |
| | Sums | | | | | | | | |
| Irritation Score | | | | | | | | | |
| | | L20 | | L21 | | | | | |
| Intact | 24 | Corrosive* | | Corrosive* | | | | | |
| Abraded | 24 | | | | | | | | |
| Intact | 72 | | | | | | | | |
| Intact | 72 | | | | | | | | |
| Abraded | 72 | | | | | | | | |
| | Sums | | | | | | | | |
| Irritation Score | | | | | | | | | |

*Irritation scores are not applicable to corrosive substances, since corrosion is more severe than the highest scored irritations.

TABLE 3

Eye Irritation Induced in Rabbits by Placing the Water in Contact with the Ocular Tissue; Responses Scored According to Procedural Regulations at the Times Specified

| Ident. No. of Sample | Lapsed Hours 24 | | | | 48 | | | | 72 | | | | Total Score | Number of Rabbits Judged Positive |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R | Ch | C | I* | R | Ch | C | I* | R | Ch | C | I* | | |
| S56 | 14 | 7 | 9 | 2 | 9 | 6 | 10 | 4 | 6 | 2 | 6 | 1 | 76 | 6 |
| S57 | 10 | 2 | 2 | 0 | 4 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 21 | 3 |
| S58 | 11 | 4 | 4 | 1 | 7 | 3 | 2 | 2 | 2 | 0 | 0 | 0 | 36 | 4 |
| S59 | 4 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 |
| S60 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 10 | 0 |
| S61 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 4 | 0 |
| S62 | 5 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 12 | 1 |
| L15 | 21 | 12 | 12 | 4 | 14 | 10 | 11 | 2 | 7 | 5 | 6 | 1 | 105 | 6 |
| L16 | Insufficient volume of sample to conclude the test. | | | | | | | | | | | | | |
| L17 | 19 | 12 | 6 | 3 | 7 | 4 | 1 | 0 | 2 | 1 | 0 | 0 | 55 | 6 |
| L18 | 10 | 3 | 4 | 1 | 6 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 34 | 3 |
| L19 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 3 | 0 |
| L20 | 3 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 |
| L21 | 18 | 16 | 12 | 6 | 10 | 16 | 12 | 6 | 10 | 16 | 12 | 5 | 139 | 6 |

*R=redness, Ch-chemosis (edems) in the palebral conjunctiva; C=corneal opacity; I=irritation response in the iris.

TABLE 4A

The Immediate Toxicity of Specific Water Samples When Given Orally to Mice (Lot M17)

| | Sample Dosage ml/Kg | Deaths (10 mice per dose) | 0 | 3 | 7 | 14 | Change in Weight % |
|---|---|---|---|---|---|---|---|
| S56 | 10 | | 36.7 | 36.4 | 37.3 | 38.0 | 3.7 |
| | 25 | | 34.4 | 33.1 | 33.9 | 35.2 | 2.1 |
| | 40 | 2 | 35.4 | 35.6 | 33.1 | 34.6 | -2.2 |
| | 63 | 9 | 34.4 | 34.0 | 32.5 | 34.5 | 0.2 |
| | 100 | 10 | 35.6 | - | - | - | - |
| Average | | | 35.3 | 34.1 | 34.8 | 35.9 | 1.9 |
| S57 | 10 | | 36.4 | 36.1 | 37.0 | 37.9 | 3.9 |
| | 25 | | 35.9 | 35.1 | 35.4 | 37.5 | 4.5 |
| | 40 | 4 | 35.1 | 32.1 | 33.4 | 35.7 | 1.6 |
| | 63 | 7 | 35.3 | 33.8 | 34.2 | 36.2 | 2.5 |
| | 100 | 10 | 34.5 | - | - | - | - |
| Average | | | 35.4 | 34.8 | 35.4 | 37.1 | 4.7 |
| S61 | 10 | | 35.1 | 35.4 | 36.4 | 36.9 | 5.2 |
| | 25 | | 32.7 | 32.7 | 33.2 | 34.6 | 5.8 |
| | 40 | | 34.0 | 33.6 | 34.4 | 35.0 | 2.8 |
| | 63 | | 33.9 | 33.8 | 34.8 | 34.4 | 1.4 |
| | 100 | 1 | 33.6 | 33.6 | 34.6 | 33.4 | -0.7 |
| Average | | | 33.9 | 33.8 | 34.7 | 34.9 | 3.0 |
| S62 | 10 | | 35.6 | 34.5 | 35.4 | 36.4 | 2.4 |
| | 25 | | 37.4 | 36.8 | 37.1 | 39.3 | 5.0 |
| | 40 | | 33.2 | 32.7 | 33.2 | 35.5 | 6.7 |
| | 63 | | 34.5 | 34.4 | 35.2 | 35.9 | 4.1 |
| | 100 | 1 | 34.6 | 34.0 | 34.8 | 36.7 | 6.2 |
| Average | | | 35.1 | 34.5 | 35.1 | 36.8 | 4.9 |
| L15 | 10 | | 33.7 | 32.6 | 33.6 | 35.0 | 4.0 |
| | 25 | | 33.3 | 31.3 | 32.4 | 35.0 | 5.0 |
| | 40 | 9 | 34.9 | 28.0 | 29.5 | 32.5 | -6.9 |
| | 63 | 10 | 34.4 | - | - | - | - |
| | 100 | 10 | 34.3 | - | - | - | - |
| Average | | | 34.1 | 31.7 | 32.8 | 34.9 | 2.2 |

TABLE 4B

The Immediate Toxicity of Specific Water Samples When Given Orally to Mice (Lot M18)

| | Sample Dosage ml/kg | Deaths (10 mice per dose) | 0 | 3 | 7 | 14 | Change in Weight % |
|---|---|---|---|---|---|---|---|
| S58- | 10 | | 30.0 | 29.4 | 30.2 | 32.1 | 6.9 |
| | 25 | | 32.2 | 32.8 | 33.7 | 35.1 | 9.0 |
| | 40 | 1 | 32.4 | 31.9 | 32.9 | 35.1 | 8.1 |
| | 63 | 10 | 32.1 | - | - | - | - |
| | 100 | 8/8* | 30.3 | - | - | - | - |
| Average | | | 31.4 | 31.3 | 32.2 | 34.0 | 8.3 |
| S60- | 10 | | 30.5 | 29.9 | 31.6 | 33.2 | 8.8 |
| | 25 | | 30.7 | 30.5 | 31.5 | 32.8 | 6.8 |
| | 40 | | 31.3 | 32.2 | 32.4 | 33.9 | 8.0 |
| | 63 | | 31.5 | 31.2 | 32.4 | 34.0 | 7.7 |
| | 100 | | 30.7 | 30.4 | 31.7 | 33.4 | 8.8 |
| Average | | | 30.9 | 30.8 | 31.9 | 33.4 | 8.0 |
| L16- | 10 | | 30.6 | 29.4 | 30.7 | 32.3 | 5.4 |
| | 25 | 1 | 29.8 | 28.1 | 29.7 | 31.8 | 6.7 |
| | 40 | 1 | 33.1 | 30.2 | 30.8 | 33.3 | 0.5 |
| | 63 | 6 | 32.0 | 28.3 | 31.1 | 34.6 | 8.2 |
| | 100 | 10 | 31.0 | - | - | - | - |
| Average | | | 31.3 | 31.1 | 32.5 | 34.9 | 11.4 |
| L20- | 10 | | 31.5 | 31.3 | 32.6 | 33.6 | 6.4 |
| | 25 | 10 | 32.3 | - | - | - | - |
| | 40 | 9/9* | 32.0 | - | - | - | - |
| | 63 | 9/9* | 31.9 | - | - | - | - |
| | 100 | 8/8* | 33.2 | - | - | - | - |
| Average | | | 32.2 | 31.3 | 32.6 | 33.6 | 4.3 |
| L21- | 10 | | 32.0 | 32.1 | 32.8 | 32.3 | 0.9 |
| | 25 | 3 | 32.9 | 32.2 | 33.9 | 34.9 | 6.1 |
| | 40 | 10 | 32.0 | - | - | - | - |
| | 63 | 10 | 33.8 | - | - | - | - |
| | 100 | 5/5 | 32.6 | - | - | - | - |
| Average | | | 32.7 | 32.1 | 33.2 | 33.4 | 2.1 |

*There was not enough solution to treat 10 animals

TABLE 4C

The Immediate Toxicity of Specific Water Samples When Given Orally to Mice. (Lot M19)

| | Sample Dosage ml/kg | Deaths (10 mice per dose) | 0 | 3 | 7 | 14 | Change in Weight % |
|---|---|---|---|---|---|---|---|
| L17 | 10 | | 24.8 | 25.3 | 28.0 | 30.4 | 22.3 |
| | 25 | 0/9* | 25.8 | 24.6 | 27.2 | 27.7 | 7.1 |
| | 40 | 0/9* | 25.4 | 26.2 | 28.8 | 30.7 | 21.0 |
| | 63 | 0/8* | 24.1 | 23.9 | 25.6 | 28.6 | 19.0 |
| | 100 | 8/8* | 23.6 | - | - | - | - |
| Average | | | 24.8 | 25.0 | 27.4 | 29.4 | 18.6 |
| L18 | 10 | | 23.5 | 23.9 | 26.5 | 28.8 | 22.4 |
| | 25 | 0/8* | 25.0 | 25.0 | 26.4 | 28.3 | 13.4 |
| | 40 | 2/8* | 25.7 | 24.4 | 26.6 | 30.6 | 19.1 |
| | 63 | 8/8* | 25.7 | - | - | - | - |
| | 100 | 8/8* | 24.5 | - | - | - | - |
| Average | | | 24.8 | 24.4 | 26.5 | 29.1 | 17.2 |
| L19 | 10 | | 23.4 | 24.4 | 27.3 | 29.8 | 26.9 |
| | 25 | 3 | 25.1 | 24.9 | 27.1 | 30.0 | 19.7 |
| | 40 | | 23.7 | 23.2 | 25.0 | 27.7 | 16.8 |
| | 63 | 4 | 24.6 | 23.3 | 24.2 | 27.8 | 12.7 |
| | 100 | 10 | 23.7 | - | - | - | - |
| Average | | | 24.1 | 26.3 | 28.6 | 31.7 | 31.5 |
| S59 | 10 | | 24.3 | 24.8 | 27.1 | 29.7 | 22.0 |
| | 25 | 0/9* | 24.3 | 25.4 | 27.4 | 30.9 | 27.1 |
| | 40 | 0/9* | 24.8 | 25.3 | 27.3 | 30.1 | 21.0 |
| | 63 | 0/9* | 24.3 | 24.6 | 26.2 | 29.8 | 22.7 |
| | 100 | 2/9* | 24.3 | 22.9 | 25.4 | 29.2 | 20.4 |
| Average | | | 24.4 | 24.7 | 26.7 | 30.0 | 22.8 |

*There was not enough solution to treat 10 animals.

TABLE 5

Summary of Results

| Water Ident. Number | Irritation Scores: Eye Sum | Irritation Scores: Skin N[a] | Irritation Scores: Skin Average Score | Mortality[b] | Average Wt.(g) all doses (days after dose): 0 | 3 | 7 | 14 | Change in wt. Percent |
|---|---|---|---|---|---|---|---|---|---|
| S56 | 76 | 6 | Corrosive[c] | | 35.3 | 34.1 | 34.8 | 35.9 | 1.9 |
| S57 | 21 | 3 | Corrosive | | 35.4 | 34.8 | 35.4 | 37.1 | 4.7 |
| S58 | 36 | 4 | Corrosive | | 31.4 | 31.3 | 32.2 | 34.0 | 8.3 |
| S59 | 5 | 0 | Corrosive | | 24.4 | 24.7 | 26.7 | 30.0 | 22.8 |
| S60 | 10 | 0 | 0.98 | | 30.9 | 30.8 | 31.9 | 33.4 | 8.0 |
| S61 | 4 | 0 | 1.13 | | 33.9 | 33.8 | 34.7 | 34.9 | 3.0 |
| S62 | 12 | 1 | 1.15 | | 35.1 | 34.5 | 35.1 | 36.8 | 4.9 |
| L15 | 105 | 6 | 5.40 | | 34.1 | 31.7 | 32.8 | 34.9 | 2.2 |
| L16 | Insufficient volume of sample | | | | 31.3 | 31.1 | 32.5 | 34.9 | 11.4 |
| L17 | 55 | 6 | Corrosive | | 24.8 | 25.0 | 27.4 | 29.4 | 18.6 |
| L18 | 34 | 3 | Corrosive | | 24.8 | 24.4 | 26.5 | 29.1 | 17.2 |
| L19 | 3 | 0 | Corrosive | | 24.1 | 26.3 | 28.6 | 31.7 | 31.5 |
| L20 | 4 | 0 | Corrosive | | 32.2 | 31.3 | 32.6 | 33.6 | 4.3 |
| L21 | 139 | 6 | Corrosive | | 32.7 | 32.1 | 33.2 | 33.4 | 2.1 |

(a) Number judged positive
(b) Mortality data for mice listed in Table 4 and Figure 1.
(c) Irritation scores are not applicable to corrosive substances since corrosion is more severe than the highest scored irritations.

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# THE TOXICITY AND IRRITANCY OF ULTRAFILTRATES OF NON-SANITARY MILITARY WASTES

Progress Report for
June 1, - September 20, 1976

Sylvan Witherup, B.S.

September 20, 1976

Supported by

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## TABLE OF CONTENTS

## Introduction

This report contains data pertaining to the irritant properties of the nine water samples described in Table 1, and which continue in consecutive order from those described in the Progress Report for April 1, - May 31, 1976 (June 8, 1976). The immediate oral toxicity tests were not performed because of insufficient volumes of the samples.

## Results

### Skin Irritation

Table 2 lists the average scores for erythema and for edema in the intact and abraded abdominal skin of six rabbits, immediately following 24 hours of contact with the respective waters, and 48 hours after the contact was terminated. Six of the eight samples tested (S-63, S-64, S-65, L-22, L-24 and L-25) were corrosive, causing necrosis of the skin, and since corrosion is more severe than irritation, scores for these samples are not applicable. The small volume of S-66 was insufficient for skin tests. The other samples, L-23 and L-26, had average scores of 2.75 and 3.48, respectively.

### Eye Irritation

A summary of the eye irritation scores is provided in Table 3. The severity and extent of the responses were sufficient to classify samples L-22, L-24 and L-25 as eye irritants. Irritation responses to these consisted of severe erythema and severe chemosis of the palpebral conjunctiva. Positive responses in the cornea and in the iris were also noted. Responses induced by sample L-24 were particularly severe.

Except or three animals that exhibited #2 degree redness at the 24-hour reading (two treated with 563, one with 565), eye irritations caused by the remaining six samples consisted of no more than mild palpebral erythema. There

was no chemosis in the conjunctivae and no involvement of the cornea or iris.

## Summary

Nine water samples have been tested with respect to their irritancy. The observations are summarized in Table 4.

Samples L-22, L-24 and L-25 caused severe physiological changes in both the skin and the eyes of rabbits. Samples S-63, S-64 and S-65 were corrosive to the skin of rabbits, but produced only mild irritation in their eyes. Samples L-23 and L-26 produced very mild reactions in the eyes of rabbits and were mildly irritating to the skin, but insufficient to classify as a primary skin irritant. Sample 566 caused mild irritation in rabbit eyes; because of its short supply, it was not tested for skin irritancy. None of the nine samples was tested for immediate oral toxicity.

### Table 1

### Identification of the Waters Studied

| | |
|---|---|
| S63 | Unfiltered Concentrated Synthetic Shower Waste (S56 diluted 1:4) |
| S64 | Unfiltered Concentrated Synthetic Shower Waste (S57 diluted 1:4) |
| S65 | Concentrated Synthetic Shower Waste |
| S66 | Concentrated Synthetic Shower Waste Filtered (S59 diluted 1:2) |
| L22 | Concentrated Laundry Waste, Filtered (L15 diluted 1:3) |
| L23 | Concentrated Army Detergent (L20 diluted 1:2) |
| L24 | Concentrated Synthetic Laundry Waste |
| L25 | Concentrated Synthetic Laundry Waste |
| L26 | Concentrated Synthetic Laundry Waste |

Table 2

Primary Irritation Induced in the Skin of Rabbits;
Scored According to Procedural Regulations

| Condition of the Skin | Time (hrs) | Average Score (6 Rabbits) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema | Ery-thema | Edema |
| | | S63 | | S64 | | S65 | | L22 | |
| Intact | 24 | Corrosive* | | Corrosive* | | Corrosive* | | Corrosive* | |
| Abraded | 24 | | | | | | | | |
| Intact | 72 | | | | | | | | |
| Abraded | 72 | | | | | | | | |
| | Sums | | | | | | | | |
| Irritation Score | | | | | | | | | |
| | | L23 | | L24 | | L25 | | L26 | |
| Intact | 24 | 2.0 | 1.2 | | | | | 1.2 | 0.7 |
| Abraded | 24 | 3.0 | 2.8 | Corrosive* | | Corrosive* | | 3.7 | 3.5 |
| Intact | 72 | 0.5 | 0.0 | | | | | 0.0 | 0.0 |
| Abraded | 72 | 1.3 | 0.2 | | | | | 3.3 | 1.5 |
| | Sums | 6.8 | 4.2 | | | | | 8.2 | 5.7 |
| Irritation Score | | 2.75 | | | | | | 3.48 | |

*Irritation scores are not applicable to corrosive substances since corrosion is more severe than the highest scored irritations.

Table 3

Eye Irritation Induced in Rabbits by Placing the Water in Contact with the Ocular Tissue; Responses Scored According to Procedural Regulations at the Times Specified.

| Ident. No. of Sample | Lapsed Hours 24 | | | | 48 | | | | 72 | | | | Total Score | Number of Rabbits Judged Positive |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R | Ch | C | I* | R | Ch | C | I* | R | Ch | C | I* | | |
| S63 | 8 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 17 | 2 |
| S64 | 6 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 10 | 0 |
| S65 | 6 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 11 | 1 |
| S66 | 4 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 9 | 0 |
| L22 | 12 | 8 | 2 | 1 | 6 | 3 | 0 | 0 | 2 | 1 | 0 | 0 | 35 | 4 (a) |
| L23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| L24 | 18 | 24 | 12 | 6 | 16 | 23 | 12 | 6 | 14 | 18 | 7 | 3 | 159 | 6 (a) |
| L25 | 12 | 11 | 2 | 1 | 6 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 36 | 5 (a) |
| L26 | 6 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 |

*R = redness, Ch = chemosis (edema) in the palpebral conjunctiva, C = corneal opacity, I - irritation response in the iris.

(a) sufficient to classify as an eye irritant.

Table 4

Summary of Results

| Water Ident. Number | Irritation Scores | | |
|---|---|---|---|
| | Eye Sum | N[a] | Skin Aver. Score |
| S63 | 17 | 2 | Corrosive[b] |
| S64 | 10 | 0 | Corrosive |
| S65 | 11 | 1 | Corrosive |
| S66 | 9 | 0 | (c) |
| L22 | 35 | 4* | Corrosive |
| L23 | 0 | 0 | 2.75 |
| L24 | 159 | 6* | Corrosive |
| L25 | 36 | 5* | Corrosive |
| L26 | 7 | 0 | 3.48 |

(a) Number judged positive; * Classified as an eye irritant.

(b) Irritation scores are not applicable to corrosive substances, since corrosion is more severe than the highest scored irritations.

(c) Insufficient volume of sample to perform test.

## APPENDIX C - Reports of Mutagenicity Evaluations

## 6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and the cells are incubated in the overlay for 2 to 3 days, and a few cell divisions occur during the incubation period, the test is semi-quantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test:

- The small number of cell divisions permits potential mutagens to act on replicating DNA, which is often more sensitive than nonreplicating DNA.
- The combined incubation of the compound and the cells in the overlay permits constant exposure of the indicator cells for 2 to 3 days.

### A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as that on the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs several doses ranging over two or three log concentrations, the highest of these doses being selected to show slight toxicity as determined by subjective criteria.

### B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. A factor that might modify dose-response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced, and the compound will not appear to be mutagenic.

6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS (Continued)

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct-acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar together with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Interpretation of Results

The demonstration of dose-related increases in mutant counts is the most reliable method to demonstrate mutagenicity. Mutant increases at only one or two doses may be significant if they occur at the higher doses. Increases at low or intermediate concentrations followed by reduced mutant counts at higher doses may indicate that the test chemical has a narrow activity range or that the high dose levels were toxic and the induced revertant cells were killed. We are able to detect the latter possibility by inspecting the background growth, and the former possibility can be investigated by looking at a narrow series of dose levels bracketing the presumptive active range.

It is difficult to detect mutagens with little or no toxicity in this assay since such agents are generally weak mutagens and produce only two to threefold increases in mutant counts. Variations of two to threefold are often within normal fluctuations of the spontaneous counts, and the use of even higher concentrations is often difficult because of the likelihood of overloading the system with large quantities of the chemical. To resolve the mutagenicity of such a chemical, other assays to which statistical evaluations can be applied may be necessary.

E. Relationship Between Mutagenicity and Carcinogenicity

It must be emphasized that the Ames Salmonella/microsome test is not a definitive test for chemical carcinogens. It is recognized, however, that correlative and functional relationships have been demonstrated between these two end points. The results of comparative tests on 300 chemicals by McCann et al., (Proc. Nat. Acad. Sci. USA, 72:5135-5139, 1975) show an extremely good correlation between results of microbial mutagenesis tests and in vivo rodent carcinogenesis assays.

All evaluation and interpretation of the data presented in this report are based only on the demonstration of or lack of mutagenic activity. Implications of potential for carcinogenicity cannot be made without additional evaluation.

# MUTAGENICITY EVALUATION

# OF

# S26 SYNTHETIC SHOWER WASTE

## FINAL REPORT

**SUBMITTED TO**

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
WASHINGTON, D. C. 20314

**SUBMITTED BY**

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 2683

AUGUST 27, 1976

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SPONSOR: U. S. Army Medical Research and Development Command

MATERIAL: S26 Synthetic Shower Waste

1. NAME OR CODE NUMBER: S26 Synthetic Shower Waste
2. DATE RECEIVED: June 9, 1976
3. DESCRIPTION: Straw Colored Soapy Liquid

SUBJECT: FINAL REPORT MUTAGENICITY PLATE ASSAY

# MUTAGENICITY PLATE ASSAY

## 1. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

## 2. MATERIALS

### A. Indicator Microorganisms

*Salmonella typhimurium*, strains: TA-1535, TA-98, TA-1537, TA-100, TA-1538

*Saccharomyces cerevisiae*, strain: D4

### B. Activation System (Ames *et al.*, Mutation Research 31:347, 1975)

#### 1. Reaction Mixture

| Component | Final Concentration/ml |
|---|---|
| TPN | 4 µmoles |
| Glucose-6-Phosphate | 5 µmoles |
| Sodium Phosphate | 100 µmoles |
| $MgCl_2$ | 8 µmoles |
| KCl | 33 µmoles |
| Homogenate fraction equivalent to 25 mg of wet tissue | 0.1-0.15 ml 9,000 x g supernatant of rat liver |

#### 2. S-9 Homogenate

A 9,000 x *g* supernatant was prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 five days prior to kill.

# MUTAGENICITY PLATE ASSAY

## 1. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

## 2. MATERIALS

### A. Indicator Microorganisms

*Salmonella typhimurium*, strains: TA-1535, TA-98, TA-1537, TA-100, TA-1538

*Saccharomyces cerevisiae*, strain: D4

### B. Activation System (Ames *et al.*, Mutation Research 31:347, 1975)

#### 1. Reaction Mixture

| Component | Final Concentration/ml |
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| Sodium Phosphate | 100 µmoles |
| $MgCl_2$ | 8 µmoles |
| KCl | 33 µmoles |
| Homogenate fraction equivalent to 25 mg of wet tissue | 0.1-0.15 ml 9,000 x g supernatant of rat liver |

#### 2. S-9 Homogenate

A 9,000 x g supernatant was prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 five days prior to kill.

## 2. MATERIALS (Continued)

### C. Positive Control Chemicals

Table 1 below lists the chemicals used for positive controls in the nonactivation and activation assays.

TABLE 1

| ASSAY | CHEMICAL[a] | SOLVENT | PROBABLE MUTAGENIC SPECIFICITY |
|---|---|---|---|
| Nonactivation | Methylnitrosoguanidine (MNNG) | Water or Saline | BPS[b] |
| | 2-Nitrofluorene (NF) | Dimethylsulfoxide[c] | FS[b] |
| | Quinacrine mustard (QM) | Water or saline | FS[b] |
| Activation | 2-Anthramine (ANTH) | Dimethylsulfoxide[c] | BPS[b] |
| | 2-Acetylaminofluorene (AAF) | Dimethylsulfoxide[c] | FS[b] |
| | 8-Aminoquinoline (AMQ) | Dimethylsulfoxide[c] | FS[b] |
| | Dimethylnitrosamine (DMNA) | Saline | BPS[b] |

[a]Concentrations given in Results Section

[b]BPS = Base-pair substitution
FS = Frameshift

[c]Previously shown to be nonmutagenic

### D. Solvent

Either deionized water or dimethylsulfoxide (DMSO) was used to prepare stock solutions of solid materials. All dilutions of test materials were made in either deionized water or DMSO. The solvent employed is recorded in the Results Section.

## 3. EXPERIMENTAL DESIGN

### A. Plate Test (Overlay Method)

Approximately $10^8$ cells from an overnight culture of each indicator strain were added to separate test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For non-activation tests, the four dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests four dose levels of the test chemical were added to the appropriate tubes with cells. Just prior to pouring, an aliquot of reaction mixture (0.5 ml containing the 9,000 x g liver homogenate) was added to each of the activation overlay tubes, which were then mixed, and the contents poured over the surface of a minimal agar plate and allowed to solidify. The plates were incubated for 48 hours at 37C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation were run with each assay.

### B. Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were analyzed in a computer program and reported on a printout. The results are presented as revertants per plate for each indicator strain employed in the assay. The positive and the solvent controls are provided as reference points. Other relevant data are provided on the computer printout.

4. SUMMARY OF PLATE TEST RESULTS

a. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: S26 SYNTHETIC SHOWER WASTE
b. SOLVENT: DEIONIZED WATER
c. TEST DATE: AUG. 10, 1976
NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) PER PLATE.

| TEST | SPECIES | TISSUE | REVERTANTS PER PLATE TA-1535 | TA-1537 | TA-1538 | TA-98 | TA-100 | DA* |
|---|---|---|---|---|---|---|---|---|
| NONACTIVATION | | | | | | | | |
| SOLVENT CONTROL | --- | --- | 13 | 28 | 15 | 57 | 126 | 50 |
| POSITIVE CONTROL** | --- | --- | > 1000 | > 1000 | >1000 | 800 | >1000 | 357 |
| TEST COMPOUND | | | | | | | | |
| 1.00000 UL | --- | --- | 10 | 14 | 10 | 48 | 129 | 44 |
| 5.00000 UL | --- | --- | 10 | 21 | 14 | 55 | 154 | 29 |
| 10.00000 UL | --- | --- | 8 | 25 | 11 | 46 | 169 | 35 |
| 100.00000 UL | --- | --- | 8 | 16 | 11 | 43 | 109 | 45 |
| ACTIVATION | | | | | | | | |
| SOLVENT CONTROL | RAT | LIVER | 28 | 53 | 20 | 39 | 279 | 37 |
| POSITIVE CONTROL*** | RAT | LIVER | 272 | 493 | > 1000 | >1000 | > 1000 | 41 |
| TEST COMPOUND | | | | | | | | |
| 1.00000 UL | RAT | LIVER | 45 | 33 | 20 | 36 | 266 | 36 |
| 5.00000 UL | RAT | LIVER | 25 | 34 | 17 | 38 | 287 | 38 |
| 10.00000 UL | RAT | LIVER | 32 | 37 | 19 | 37 | 253 | 32 |
| 100.00000 UL | RAT | LIVER | 28 | 35 | 22 | 37 | 230 | 34 |

* INV. CONVENTANTS PER PLATE

** TA-1535 SODIUM AZIDE 10 UG/PLATE
TA-1537 9-AA 10 UG/PLATE
TA-1538 NF 100 UG/PLATE
TA-98 NF 100 UG/PLATE
TA-100 SODIUM AZIDE 10 UG/PLATE
DA SODIUM AZIDE 10 UG/PLATE

*** TA-1535 ANTH 100 UG/PLATE
TA-1537 AMO 100 UG/PLATE
TA-1538 AAF 100 UG/PLATE
TA-98 AAF 100 UG/PLATE
TA-100 ANTH 100 UG/PLATE
DA DMNA 100 MICROMOLES/PLATE

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## 5. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound was examined for mutagenic activity in a series of *in vitro* microbial assays employing *Salmonella* and *Saccharomyces* indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats. The following results were obtained:

### A. Toxicity

The compound was tested over a series of concentrations such that there was either quantitative or qualitative evidence of some chemically induced physiological effects at the high dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of this compound was from 1 µl to 100 µl per plate.

### B. Nonactivation Test Results

The results of the tests conducted on the compound in the absence of a metabolic system were all negative.

### C. Activation Test Results

The results of the tests conducted on the compound in the presence of the rat liver activation system were all negative.

### D. Conclusions

The test compound S26 Synthetic Shower Waste did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.

Submitted by:

David Brusick, Ph.D.
Director
Department of Genetics

Reviewed by:

Robert J. Weir, Ph.D.
Vice President

# MUTAGENICITY EVALUATION

# OF

# S26A SYNTHETIC SHOWER WASTE

## FINAL REPORT

**SUBMITTED TO**

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
WASHINGTON, D. C. 20314

**SUBMITTED BY**

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 2683

AUGUST 27, 1976

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SPONSOR: U. S. Army Medical Research and Development Command

MATERIAL: S26A Synthetic Shower Waste

1. NAME OR CODE NUMBER: S26A Synthetic Shower Waste
2. DATE RECEIVED: June 9, 1976
3. DESCRIPTION: Cloudy Soapy Liquid

SUBJECT: FINAL REPORT MUTAGENICITY PLATE ASSAY

# MUTAGENICITY PLATE ASSAY

## 1. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

## 2. MATERIALS

### A. Indicator Microorganisms

*Salmonella typhimurium*, strains: TA-1535, TA-98, TA-1537, TA-100, TA-1538

*Saccharomyces cerevisiae*, strain: D4

### B. Activation System (Ames *et al.*, Mutation Research 31:347, 1975)

#### 1. Reaction Mixture

| Component | Final Concentration/ml |
|---|---|
| TPN | 4 µmoles |
| Glucose-6-Phosphate | 5 µmoles |
| Sodium Phosphate | 100 µmoles |
| $MgCl_2$ | 8 µmoles |
| KCl | 33 µmoles |
| Homogenate fraction equivalent to 25 mg of wet tissue | 0.1-0.15 ml 9,000 x g supernatant of rat liver |

#### 2. S-9 Homogenate

A 9,000 x g supernatant was prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 five days prior to kill.

## 2. MATERIALS (Continued)

### C. Positive Control Chemicals

Table 1 below lists the chemicals used for positive controls in the nonactivation and activation assays.

TABLE 1

| ASSAY | CHEMICAL[a] | SOLVENT | PROBABLE MUTAGENIC SPECIFICITY |
|---|---|---|---|
| Nonactivation | Methylnitrosoguanidine (MNNG) | Water or Saline | BPS[b] |
| | 2-Nitrofluorene (NF) | Dimethylsulfoxide[c] | FS[b] |
| | Quinacrine mustard (QM) | Water or saline | FS[b] |
| Activation | 2-Anthramine (ANTH) | Dimethylsulfoxide[c] | BPS[b] |
| | 2-Acetylaminofluorene (AAF) | Dimethylsulfoxide[c] | FS[b] |
| | 8-Aminoquinoline (AMQ) | Dimethylsulfoxide[c] | FS[b] |
| | Dimethylnitrosamine (DMNA) | Saline | BPS[b] |

[a]Concentrations given in Results Section

[b]BPS = Base-pair substitution
FS = Frameshift

[c]Previously shown to be nonmutagenic

### D. Solvent

Either deionized water or dimethylsulfoxide (DMSO) was used to prepare stock solutions of solid materials. All dilutions of test materials were made in either deionized water or DMSO. The solvent employed is recorded in the Results Section.

## 3. EXPERIMENTAL DESIGN

### A. Plate Test (Overlay Method)

Approximately $10^8$ cells from an overnight culture of each indicator strain were added to separate test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For non-activation tests, the four dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests four dose levels of the test chemical were added to the appropriate tubes with cells. Just prior to pouring, an aliquot of reaction mixture (0.5 ml containing the 9,000 x g liver homogenate) was added to each of the activation overlay tubes, which were then mixed, and the contents poured over the surface of a minimal agar plate and allowed to solidify. The plates were incubated for 48 hours at 37C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation were run with each assay.

### B. Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were analyzed in a computer program and reported on a printout. The results are presented as revertants per plate for each indicator strain employed in the assay. The positive and the solvent controls are provided as reference points. Other relevant data are provided on the computer printout.

## 4. SUMMARY OF PLATE TEST RESULTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: S26A SYNTHETIC SHOWER WASTE
B. SOLVENT: DEIONIZED WATER
C. TEST DATE: AUG. 10, 1976
NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) PER PLATE.

| TEST | SPECIES | TISSUE | REVERTANTS PER PLATE | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | TA-1535 | TA-1537 | TA-1538 | TA-98 | TA-100 | D4* |
| **NONACTIVATION** | | | | | | | | |
| SOLVENT CONTROL | --- | --- | 13 | 28 | 15 | 50 | 126 | 50 |
| POSITIVE CONTROL** | --- | --- | >1000 | >1000 | >1000 | 800 | >1000 | 357 |
| TEST COMPOUND | | | | | | | | |
| 1.00000 UL | --- | --- | 8 | 28 | 15 | 62 | 141 | 42 |
| 5.00000 UL | --- | --- | 10 | 30 | 11 | 46 | 137 | 41 |
| 10.00000 UL | --- | --- | 6 | 35 | 14 | 34 | 142 | 22 |
| 100.00000 UL | --- | --- | 5 | 13 | 11 | 45 | 96 | 38 |
| **ACTIVATION** | | | | | | | | |
| SOLVENT CONTROL | RAT | LIVER | 28 | 58 | 20 | 39 | 279 | 37 |
| POSITIVE CONTROL*** | RAT | LIVER | 272 | 493 | >1000 | >1000 | >1000 | 41 |
| TEST COMPOUND | | | | | | | | |
| 1.00000 UL | RAT | LIVER | 42 | 35 | 27 | 34 | 265 | 39 |
| 5.00000 UL | RAT | LIVER | 30 | 41 | 15 | 34 | 328 | 34 |
| 10.00000 UL | RAT | LIVER | 48 | 34 | 18 | 37 | 306 | 33 |
| 100.00000 UL | RAT | LIVER | 32 | 30 | 15 | 46 | 267 | 35 |

* 12% CONVENTANTS PER PLATE

** TA-1535 MNNG 10 UG/PLATE
TA-1537 9AM 10 UG/PLATE
TA-1538 NF 100 UG/PLATE
TA-98 NF 100 UG/PLATE
TA-100 MNNG 10 UG/PLATE
D4 MNNG 10 UG/PLATE

*** TA-1535 ANTH 100 UG/PLATE
TA-1537 AMQ 100 UG/PLATE
TA-1538 AAF 100 UG/PLATE
TA-98 AAF 100 UG/PLATE
TA-100 ANTH 100 UG/PLATE
D4 DMNA 100 MICROMOLES/PLATE

## 5. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound was examined for mutagenic activity in a series of *in vitro* microbial assays employing *Salmonella* and *Saccharomyces* indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats. The following results were obtained:

### A. Toxicity

The compound was tested over a series of concentrations such that there was either quantitative or qualitative evidence of some chemically induced physiological effects at the high dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of this compound was from 1 µl to 100 µl per plate.

### B. Nonactivation Test Results

The results of the tests conducted on the compound in the absence of a metabolic system were all negative.

### C. Activation Test Results

The results of the tests conducted on the compound in the presence of the rat liver activation system were all negative.

### D. Conclusions

The test compound S26A Synthetic Shower Waste did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.

Submitted by:

David Brusick, Ph.D.
Director
Department of Genetics

Reviewed by:

Robert S. Weir, Ph.D.
Vice President

# MUTAGENICITY EVALUATION

# OF

# S40 SYNTHETIC SHOWER WASTE

# FINAL REPORT

**SUBMITTED TO**

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
WASHINGTON, D. C. 20314

**SUBMITTED BY**

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 2683

AUGUST 27, 1976

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SPONSOR: U. S. Army Medical Research and Development Command

MATERIAL: S40 Synthetic Shower Waste

1. NAME OR CODE NUMBER: S40 Synthetic Shower Waste
2. DATE RECEIVED: June 9, 1976
3. DESCRIPTION: Greenish, Cloudy, Soapy Liquid

SUBJECT: FINAL REPORT MUTAGENICITY PLATE ASSAY

# MUTAGENICITY PLATE ASSAY

## 1. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

## 2. MATERIALS

### A. Indicator Microorganisms

*Salmonella typhimurium*, strains: TA-1535 TA-98
TA-1537 TA-100
TA-1538

*Saccharomyces cerevisiae*, strain: D4

### B. Activation System (Ames et al., Mutation Research 31:347, 1975)

#### 1. Reaction Mixture

| Component | Final Concentration/ml |
|---|---|
| TPN | 4 µmoles |
| Glucose-6-Phosphate | 5 µmoles |
| Sodium Phosphate | 100 µmoles |
| $MgCl_2$ | 8 µmoles |
| KCl | 33 µmoles |
| Homogenate fraction equivalent to 25 mg of wet tissue | 0.1-0.15 ml 9,000 x g supernatant of rat liver |

#### 2. S-9 Homogenate

A 9,000 x g supernatant was prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 five days prior to kill.

## 2. MATERIALS (Continued)

### C. Positive Control Chemicals

Table 1 below lists the chemicals used for positive controls in the nonactivation and activation assays.

TABLE 1

| ASSAY | CHEMICAL[a] | SOLVENT | PROBABLE MUTAGENIC SPECIFICITY |
|---|---|---|---|
| Nonactivation | Methylnitrosoguanidine (MNNG) | Water or Saline | BPS[b] |
| | 2-Nitrofluorene (NF) | Dimethylsulfoxide[c] | FS[b] |
| | Quinacrine mustard (QM) | Water or saline | FS[b] |
| Activation | 2-Anthramine (ANTH) | Dimethylsulfoxide[c] | BPS[b] |
| | 2-Acetylaminofluorene (AAF) | Dimethylsulfoxide[c] | FS[b] |
| | 8-Aminoquinoline (AMQ) | Dimethylsulfoxide[c] | FS[b] |
| | Dimethylnitrosamine (DMNA) | Saline | BPS[b] |

[a]Concentrations given in Results Section

[b]BPS = Base-pair substitution

FS = Frameshift

[c]Previously shown to be nonmutagenic

### D. Solvent

Either deionized water or dimethylsulfoxide (DMSO) was used to prepare stock solutions of solid materials. All dilutions of test materials were made in either deionized water or DMSO. The solvent employed is recorded in the Results Section.

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### B. Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were analyzed in a computer program and reported on a printout. The results are presented as revertants per plate for each indicator strain employed in the assay. The positive and the solvent controls are provided as reference points. Other relevant data are provided on the computer printout.

4. SUMMARY OF PLATE TEST RESULTS

a. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: S40 SYNTHETIC SHOWER GAS
b. SOLVENT: DEIONIZED WATER
c. TEST DATE: AUG. 10, 1976
NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) PER PLATE.

| TEST | SPECIES | TISSUE | REVERTANTS PER PLATE | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | TA-1535 | TA-1537 | TA-1538 | TA-98 | TA-100 | UVR* |
| **NONACTIVATION** | | | | | | | | |
| SOLVENT CONTROL | --- | --- | 14 | 28 | 15 | 57 | 126 | 50 |
| POSITIVE CONTROL** | --- | --- | >1000 | >1000 | >1000 | 800 | >1000 | 357 |
| TEST COMPOUND | | | | | | | | |
| 1.00000 UL | --- | --- | 14 | 23 | 9 | 46 | 169 | 48 |
| 5.00000 UL | --- | --- | 8 | 17 | 14 | 46 | 191 | 50 |
| 10.00000 UL | --- | --- | 10 | 26 | 12 | 53 | 168 | 35 |
| 100.00000 UL | --- | --- | 5 | 13 | 3 | 16 | 101 | 47 |
| **ACTIVATION** | | | | | | | | |
| SOLVENT CONTROL | RAT | LIVER | 28 | 58 | 20 | 39 | 279 | 37 |
| POSITIVE CONTROL*** | RAT | LIVER | 272 | 493 | >1000 | >1000 | >1000 | 41 |
| TEST COMPOUND | | | | | | | | |
| 1.00000 UL | RAT | LIVER | 37 | 39 | 27 | 54 | 287 | 38 |
| 5.00000 UL | RAT | LIVER | 30 | 36 | 17 | 38 | 302 | 32 |
| 10.00000 UL | RAT | LIVER | 26 | 34 | 24 | 35 | 245 | 35 |
| 100.00000 UL | RAT | LIVER | 15 | 39 | 17 | 41 | 157 | 37 |

* UVR = CONVENIENTS PER PLATE

** TA-1535 MMNG 10 UG/PLATE
TA-1537 9AA 10 UG/PLATE
TA-1538 NF 100 UG/PLATE
TA-98 4NQ 100 UG/PLATE
TA-100 MMNG 10 UG/PLATE
DA AMNA 10 UG/PLATE

*** TA-1535 ANTH 100 UG/PLATE
TA-1537 AMQ 100 UG/PLATE
TA-1538 AAF 100 UG/PLATE
TA-98 AAF 100 UG/PLATE
TA-100 ANTH 100 UG/PLATE
DA DMNA 100 MICROMOLES/PLATE

## 5. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound was examined for mutagenic activity in a series of in vitro microbial assays employing Salmonella and Saccharomyces indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats. The following results were obtained:

### A. Toxicity

The compound was tested over a series of concentrations such that there was either quantitative or qualitative evidence of some chemically induced physiological effects at the high dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of this compound was from 1 µl to 100 µl per plate.

### B. Nonactivation Test Results

The results of the tests conducted on the compound in the absence of a metabolic system were all negative.

### C. Activation Test Results

The results of the tests conducted on the compound in the presence of the rat liver activation system were all negative.

### D. Conclusions

The test compound S40 Synthetic Shower Waste did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.

Submitted by:

David Brusick, Ph.D.
Director
Department of Genetics

Reviewed by:

Robert J. Weir, Ph.D.
Vice President

# MUTAGENICITY EVALUATION

# OF

# S50 ACTUAL SHOWER WASTE

# FINAL REPORT

SUBMITTED TO

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
WASHINGTON, D. C. 20314

SUBMITTED BY

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 2683

AUGUST 27, 1976

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SPONSOR: U. S. Army Medical Research and Development Command

MATERIAL: S50 Actual Shower Waste

1. NAME OR CODE NUMBER: S50 Actual Shower Waste
2. DATE RECEIVED: June 9, 1976
3. DESCRIPTION: White Liquid

SUBJECT: FINAL REPORT MUTAGENICITY PLATE ASSAY

# MUTAGENICITY PLATE ASSAY

## 1. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

## 2. MATERIALS

### A. Indicator Microorganisms

*Salmonella typhimurium*, strains: TA-1535, TA-98, TA-1537, TA-100, TA-1538

*Saccharomyces cerevisiae*, strain: D4

### B. Activation System (Ames *et al.*, *Mutation Research* 31:347, 1975)

#### 1. Reaction Mixture

| Component | Final Concentration/ml |
|---|---|
| TPN | 4 µmoles |
| Glucose-6-Phosphate | 5 µmoles |
| Sodium Phosphate | 100 µmoles |
| $MgCl_2$ | 8 µmoles |
| KCl | 33 µmoles |
| Homogenate fraction equivalent to 25 mg of wet tissue | 0.1-0.15 ml 9,000 x g supernatant of rat liver |

#### 2. S-9 Homogenate

A 9,000 x g supernatant was prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 five days prior to kill.

2. MATERIALS (Continued)

C. Positive Control Chemicals

Table 1 below lists the chemicals used for positive controls in the nonactivation and activation assays.

TABLE 1

| ASSAY | CHEMICAL[a] | SOLVENT | PROBABLE MUTAGENIC SPECIFICITY |
|---|---|---|---|
| Nonactivation | Methylnitrosoguanidine (MNNG) | Water or Saline | BPS[b] |
| | 2-Nitrofluorene (NF) | Dimethylsulfoxide[c] | FS[b] |
| | Quinacrine mustard (QM) | Water or saline | FS[b] |
| Activation | 2-Anthramine (ANTH) | Dimethylsulfoxide[c] | BPS[b] |
| | 2-Acetylaminofluorene (AAF) | Dimethylsulfoxide[c] | FS[b] |
| | 8-Aminoquinoline (AMQ) | Dimethylsulfoxide[c] | FS[b] |
| | Dimethylnitrosamine (DMNA) | Saline | BPS[b] |

[a]Concentrations given in Results Section

[b]BPS = Base-pair substitution
FS = Frameshift

[c]Previously shown to be nonmutagenic

D. Solvent

Either deionized water or dimethylsulfoxide (DMSO) was used to prepare stock solutions of solid materials. All dilutions of test materials were made in either deionized water or DMSO. The solvent employed is recorded in the Results Section.

## 3. EXPERIMENTAL DESIGN

### A. Plate Test (Overlay Method)

Approximately $10^8$ cells from an overnight culture of each indicator strain were added to separate test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For non-activation tests, the four dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests four dose levels of the test chemical were added to the appropriate tubes with cells. Just prior to pouring, an aliquot of reaction mixture (0.5 ml containing the 9,000 x g liver homogenate) was added to each of the activation overlay tubes, which were then mixed, and the contents poured over the surface of a minimal agar plate and allowed to solidify. The plates were incubated for 48 hours at 37C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation were run with each assay.

### B. Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were analyzed in a computer program and reported on a printout. The results are presented as revertants per plate for each indicator strain employed in the assay. The positive and the solvent controls are provided as reference points. Other relevant data are provided on the computer printout.

[redacted] TEST RESULTS

[redacted] CODE DESIGNATION OF THE TEST COMPOUND: S50 ACTUAL SHOWER WASTE
[redacted]: [redacted]ELDMANIZED WATER
[redacted] TEST DATE: APRIL 10, 1976
NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) PER PLATE.

| Trial | SPECIES | TISSUE | REVERTANTS PER PLATE TA-1535 | | TA-1537 | TA-1538 | TA-98 | TA-100 | D4* |
|---|---|---|---|---|---|---|---|---|---|
| [redacted] | | | | | | | | | |
| SOLVENT CONTROL | --- | --- | 14 | | 28 | 15 | 57 | 126 | 50 |
| POSITIVE CONTROL** | --- | --- | >1000 | | >1000 | >1000 | 800 | >1000 | 357 |
| TEST COMPOUND | | | | | | | | | |
| 1.00000 UL | --- | --- | 12 | | 14 | 8 | 37 | 145 | 36 |
| 5.00000 UL | --- | --- | 9 | | 20 | 11 | 35 | 158 | 46 |
| 10.00000 UL | --- | --- | 8 | | 24 | 17 | 34 | 182 | 45 |
| 100.00000 UL | --- | --- | 16 | | 20 | 12 | 45 | 164 | 40 |
| [redacted] | | | | | | | | | |
| SOLVENT CONTROL | RAT | LIVER | 28 | 11 | 53 | 20 | 39 | 279 | 37 |
| POSITIVE CONTROL*** | RAT | LIVER | 272 | | 493 | >1000 | >1000 | >1000 | 41 |
| TEST COMPOUND | | | | | | | | | |
| 1.00000 UL | RAT | LIVER | 41 | - | 46 | 21 | 31 | 263 | 33 |
| 5.00000 UL | RAT | LIVER | 43 | - | 51 | 24 | 42 | 276 | 37 |
| 10.00000 UL | RAT | LIVER | 48 | - | 33 | 27 | 41 | 296 | 38 |
| 100.00000 UL | RAT | LIVER | 82 | 9 | 37 | 21 | 32 | 271 | 34 |

* [redacted] CONCENTRATIONS PER PLATE

** TA-1535 [redacted] 10 UG/PLATE
TA-1537 [redacted] 10 UG/PLATE
TA-1538 [redacted] 100 UG/PLATE
TA-98 [redacted] 100 UG/PLATE
TA-100 [redacted] 10 UG/PLATE
D4 [redacted] 10 UG/PLATE

*** TA-1535 ANTH 100 UG/PLATE
TA-1537 AMQ 100 UG/PLATE
TA-1538 AAF 100 UG/PLATE
TA-98 AAF 100 UG/PLATE
TA-100 ANTH 100 UG/PLATE
D4 DMNA 100 MICROMOLES/PLATE

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## 5. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound was examined for mutagenic activity in a series of *in vitro* microbial assays employing *Salmonella* and *Saccharomyces* indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats. The following results were obtained:

### A. Toxicity

The compound was tested over a series of concentrations such that there was either quantitative or qualitative evidence of some chemically induced physiological effects at the high dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of this compound was from 1 µl to 100 µl per plate.

### B. Nonactivation Test Results

The results of the tests conducted on the compound in the absence of a metabolic system were all negative.

### C. Activation Test Results

The results of the tests conducted on the compound in the presence of the rat liver activation system were all negative. The 100 µl per plate dose with TA-1535 was repeated because of a slightly increased mutant frequency. The repeat test was negative.

### D. Conclusions

The test compound S50 Actual Shower Waste did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.

Submitted by:

David Brusick, Ph.D.
Director
Department of Genetics

Reviewed by:

Robert J. Weir, Ph.D.
Vice President

# MUTAGENICITY EVALUATION

# OF

# S53 ACTUAL SHOWER WASTE

# FINAL REPORT

**SUBMITTED TO**

**U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND**
**WASHINGTON, D. C. 20314**

**SUBMITTED BY**

**LITTON BIONETICS, INC.**
**5516 NICHOLSON LANE**
**KENSINGTON, MARYLAND 20795**

**LBI PROJECT NO. 2683**

**AUGUST 27, 1976**

## TABLE OF CONTENTS



SPONSOR: U. S. Army Medical Research and Development Command

MATERIAL: S53 Actual Shower Waste

1. NAME OR CODE NUMBER: S53 Actual Shower Waste
2. DATE RECEIVED: June 9, 1976
3. DESCRIPTION: Clear Liquid

SUBJECT: FINAL REPORT MUTAGENICITY PLATE ASSAY

# MUTAGENICITY PLATE ASSAY

## 1. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

## 2. MATERIALS

### A. Indicator Microorganisms

*Salmonella typhimurium*, strains: TA-1535, TA-98, TA-1537, TA-100, TA-1538

*Saccharomyces cerevisiae*, strain: D4

### B. Activation System (Ames *et al.*, Mutation Research 31:347, 1975)

#### 1. Reaction Mixture

| Component | Final Concentration/ml |
|---|---|
| TPN | 4 µmoles |
| Glucose-6-Phosphate | 5 µmoles |
| Sodium Phosphate | 100 µmoles |
| $MgCl_2$ | 8 µmoles |
| KCl | 33 µmoles |
| Homogenate fraction equivalent to 25 mg of wet tissue | 0.1-0.15 ml 9,000 x g supernatant of rat liver |

#### 2. S-9 Homogenate

A 9,000 x g supernatant was prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 five days prior to kill.

2. MATERIALS (Continued)

C. Positive Control Chemicals

Table 1 below lists the chemicals used for positive controls in the nonactivation and activation assays.

TABLE 1

| ASSAY | CHEMICAL[a] | SOLVENT | PROBABLE MUTAGENIC SPECIFICITY |
|---|---|---|---|
| Nonactivation | Methylnitrosoguanidine (MNNG) | Water or Saline | BPS[b] |
| | 2-Nitrofluorene (NF) | Dimethylsulfoxide[c] | FS[b] |
| | Quinacrine mustard (QM) | Water or saline | FS[b] |
| Activation | 2-Anthramine (ANTH) | Dimethylsulfoxide[c] | BPS[b] |
| | 2-Acetylaminofluorene (AAF) | Dimethylsulfoxide[c] | FS[b] |
| | 8-Aminoquinoline (AMQ) | Dimethylsulfoxide[c] | FS[b] |
| | Dimethylnitrosamine (DMNA) | Saline | BPS[b] |

[a]Concentrations given in Results Section

[b]BPS = Base-pair substitution
FS = Frameshift

[c]Previously shown to be nonmutagenic

D. Solvent

Either deionized water or dimethylsulfoxide (DMSO) was used to prepare stock solutions of solid materials. All dilutions of test materials were made in either deionized water or DMSO. The solvent employed is recorded in the Results Section.

## 3. EXPERIMENTAL DESIGN

### A. Plate Test (Overlay Method)

Approximately $10^8$ cells from an overnight culture of each indicator strain were added to separate test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For non-activation tests, the four dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests four dose levels of the test chemical were added to the appropriate tubes with cells. Just prior to pouring, an aliquot of reaction mixture (0.5 ml containing the 9,000 x g liver homogenate) was added to each of the activation overlay tubes, which were then mixed, and the contents poured over the surface of a minimal agar plate and allowed to solidify. The plates were incubated for 48 hours at 37C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation were run with each assay.

### B. Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were analyzed in a computer program and reported on a printout. The results are presented as revertants per plate for each indicator strain employed in the assay. The positive and the solvent controls are provided as reference points. Other relevant data are provided on the computer printout.

4. SUMMARY OF PLATE TEST RESULTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: 553 ACTUAL SHOWER WASTE
B. SOLVENT: DEIONIZED WATER
C. TEST DATE: AUG. 10, 1978
NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) PER PLATE.

| TEST | SPECIES | TISSUE | REVERTANTS PER PLATE TA-1535 | TA-1537 | TA-1538 | TA-98 | TA-100 | D4* |
|---|---|---|---|---|---|---|---|---|
| **NONACTIVATION** | | | | | | | | |
| SOLVENT CONTROL | --- | --- | 14 | 28 | 15 | 57 | 126 | 58 |
| POSITIVE CONTROL** | --- | --- | >1000 | > 1000 | > 1000 | 400 | > 1000 | 357 |
| TEST COMPOUND | | | | | | | | |
| 1.00000 UL | --- | --- | 17 | 17 | 10 | 40 | 131 | 64 |
| 5.00000 UL | --- | --- | 8 | 21 | 7 | 40 | 126 | 59 |
| 10.00000 UL | --- | --- | 18 | 22 | 11 | 47 | 173 | 48 |
| 100.00000 UL | --- | --- | 11 | 26 | 14 | 45 | 161 | 67 |
| **ACTIVATION** | | | | | | | | |
| SOLVENT CONTROL | RAT | LIVER | 28 | 58 | 20 | 39 | 279 | 37 |
| POSITIVE CONTROL*** | RAT | LIVER | 272 | 493 | > 1000 | >1000 | >1000 | 41 |
| TEST COMPOUND | | | | | | | | |
| 1.00000 UL | RAT | LIVER | 38 | 53 | 17 | 42 | 287 | 33 |
| 5.00000 UL | RAT | LIVER | 29 | 57 | 22 | 32 | 292 | 35 |
| 10.00000 UL | RAT | LIVER | 41 | 48 | 26 | 30 | 317 | 33 |
| 100.00000 UL | RAT | LIVER | 30 | 46 | 25 | 41 | 303 | 29 |

* INV. CONTAMINTS PER PLATE

** TA-1535 [illegible] 10 UG/PLATE
TA-1537 [illegible] 10 UG/PLATE
TA-1538 NF 100 UG/PLATE
TA-98 NF 100 UG/PLATE
TA-100 [illegible] 10 UG/PLATE
D4 [illegible] 10 UG/PLATE

*** TA-1535 ANTH 100 UG/PLATE
TA-1537 ANU 100 UG/PLATE
TA-1538 AAF 100 UG/PLATE
TA-98 AAF 100 UG/PLATE
TA-100 ANTH 100 UG/PLATE
D4 UMNA 100 MICROMOLES/PLATE

## 5. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound was examined for mutagenic activity in a series of *in vitro* microbial assays employing *Salmonella* and *Saccharomyces* indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats. The following results were obtained:

### A. Toxicity

The compound was tested over a series of concentrations such that there was either quantitative or qualitative evidence of some chemically induced physiological effects at the high dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of this compound was from 1 µl to 100 µl per plate.

### B. Nonactivation Test Results

The results of the tests conducted on the compound in the absence of a metabolic system were all negative.

### C. Activation Test Results

The results of the tests conducted on the compound in the presence of the rat liver activation system were all negative.

### D. Conclusions

The test compound S53 Actual Shower Waste did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.

Submitted by:

David Brusick, Ph.D.
Director
Department of Genetics

Reviewed by:

Robert J. Weir, Ph.D.
Vice President

# MUTAGENICITY EVALUATION

# OF

# S55A ACTUAL SHOWER WATER

# FINAL REPORT

**SUBMITTED TO**

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
WASHINGTON, D. C. 20314

**SUBMITTED BY**

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 2683

AUGUST 27, 1976

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SPONSOR: U. S. Army Medical Research and Development Command

MATERIAL: S55A Actual Shower Water

1. NAME OR CODE NUMBER: S55A Actual Shower Water
2. DATE RECEIVED: June 9, 1976
3. DESCRIPTION: Clear Soapy Liquid

SUBJECT: FINAL REPORT MUTAGENICITY PLATE ASSAY

## MUTAGENICITY PLATE ASSAY

### 1. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

### 2. MATERIALS

#### A. Indicator Microorganisms

*Salmonella typhimurium*, strains: TA-1535, TA-98, TA-1537, TA-100, TA-1538

*Saccharomyces cerevisiae*, strain: D4

#### B. Activation System (Ames *et al.*, Mutation Research 31:347, 1975)

1. Reaction Mixture

| Component | Final Concentration/ml |
|---|---|
| TPN | 4 µmoles |
| Glucose-6-Phosphate | 5 µmoles |
| Sodium Phosphate | 100 µmoles |
| $MgCl_2$ | 8 µmoles |
| KCl | 33 µmoles |
| Homogenate fraction equivalent to 25 mg of wet tissue | 0.1-0.15 ml 9,000 x g supernatant of rat liver |

2. S-9 Homogenate

A 9,000 x g supernatant was prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 five days prior to kill.

## 2. MATERIALS (Continued)

### C. Positive Control Chemicals

Table 1 below lists the chemicals used for positive controls in the nonactivation and activation assays.

TABLE 1

| ASSAY | CHEMICAL[a] | SOLVENT | PROBABLE MUTAGENIC SPECIFICITY |
|---|---|---|---|
| Nonactivation | Methylnitrosoguanidine (MNNG) | Water or Saline | BPS[b] |
| | 2-Nitrofluorene (NF) | Dimethylsulfoxide[c] | FS[b] |
| | Quinacrine mustard (QM) | Water or saline | FS[b] |
| Activation | 2-Anthramine (ANTH) | Dimethylsulfoxide[c] | BPS[b] |
| | 2-Acetylaminofluorene (AAF) | Dimethylsulfoxide[c] | FS[b] |
| | 8-Aminoquinoline (AMQ) | Dimethylsulfoxide[c] | FS[b] |
| | Dimethylnitrosamine (DMNA) | Saline | BPS[b] |

[a]Concentrations given in Results Section

[b]BPS = Base-pair substitution

FS = Frameshift

[c]Previously shown to be nonmutagenic

### D. Solvent

Either deionized water or dimethylsulfoxide (DMSO) was used to prepare stock solutions of solid materials. All dilutions of test materials were made in either deionized water or DMSO. The solvent employed is recorded in the Results Section.

## 3. EXPERIMENTAL DESIGN

### A. Plate Test (Overlay Method)

Approximately $10^8$ cells from an overnight culture of each indicator strain were added to separate test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For non-activation tests, the four dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests four dose levels of the test chemical were added to the appropriate tubes with cells. Just prior to pouring, an aliquot of reaction mixture (0.5 ml containing the 9,000 x g liver homogenate) was added to each of the activation overlay tubes, which were then mixed, and the contents poured over the surface of a minimal agar plate and allowed to solidify. The plates were incubated for 48 hours at 37C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation were run with each assay.

### B. Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were analyzed in a computer program and reported on a printout. The results are presented as revertants per plate for each indicator strain employed in the assay. The positive and the solvent controls are provided as reference points. Other relevant data are provided on the computer printout.

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THE TOXICITY AND IRRITANCY OF ULTRAFILTRATES OF NON-SANITARY MI--ETC(U)
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4 SUMMARY OF PLATE TEST RESULTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: S55A ACTUAL SHOWER WATER
B. SOLVENT: DEIONIZED WATER
C. TEST DATE: AUG. 10, 1976
NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) PER PLATE.

| TEST | SPECIES | TISSUE | REVERTANTS PER PLATE | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | TA-1535 | TA-1537 | TA-1538 | TA-98 | TA-100 | D4* |
| **NON-ACTIVATION** | | | | | | | | |
| SOLVENT CONTROL | --- | --- | 14 | 28 | 21 | 57 | 126 | 39 |
| POSITIVE CONTROL** | --- | --- | >1000 | >1000 | >1000 | 800 | >1000 | 357 |
| TEST COMPOUND | | | | | | | | |
| 1.00000 UL | --- | --- | 13 | 20 | 26 | 40 | 181 | 14 |
| 5.00000 UL | --- | --- | 8 | 26 | 23 | 56 | 141 | 9 |
| 10.00000 UL | --- | --- | 8 | 20 | 21 | 44 | 138 | 19 |
| 100.00000 UL | --- | --- | 12 | 21 | 22 | 40 | 150 | 26 |
| **ACTIVATION** | | | | | | | | |
| SOLVENT CONTROL | RAT | LIVER | 28 | 58 | 20 | 38 | 279 | 37 |
| POSITIVE CONTROL*** | RAT | LIVER | 272 | 493 | >1000 | >1000 | >1000 | 41 |
| TEST COMPOUND | | | | | | | | |
| 1.00000 UL | RAT | LIVER | 23 | 43 | 27 | 33 | 247 | 35 |
| 5.00000 UL | RAT | LIVER | 38 | 45 | 19 | 38 | 275 | 33 |
| 10.00000 UL | RAT | LIVER | 39 | 49 | 23 | 33 | 283 | 37 |
| 100.00000 UL | RAT | LIVER | 38 | 39 | 23 | 33 | 268 | 34 |

* DMSO CONCENTRANTS PER PLATE

** TA-1535 MNNG 10 UG/PLATE
TA-1537 9AA 10 UG/PLATE
TA-1538 NF 100 UG/PLATE
TA-98 NF 100 UG/PLATE
TA-100 MNNG 10 UG/PLATE
D4 4NNO 10 UG/PLATE

*** TA-1535 ANTH 100 UG/PLATE
TA-1537 AMQ 100 UG/PLATE
TA-1538 AAF 100 UG/PLATE
TA-98 AAF 100 UG/PLATE
TA-100 ANTH 100 UG/PLATE
D4 DMNA 100 MICROMOLES/PLATE

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## 5. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound was examined for mutagenic activity in a series of *in vitro* microbial assays employing *Salmonella* and *Saccharomyces* indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats. The following results were obtained:

### A. Toxicity

The compound was tested over a series of concentrations such that there was either quantitative or qualitative evidence of some chemically induced physiological effects at the high dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of this compound was from 1 µl to 100 µl per plate.

### B. Nonactivation Test Results

The results of the tests conducted on the compound in the absence of a metabolic system were all negative.

### C. Activation Test Results

The results of the tests conducted on the compound in the presence of the rat liver activation system were all negative.

### D. Conclusions

The test compound S55A Actual Shower Water did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.

Submitted by:

David Brusick, Ph.D.
Director
Department of Genetics

Reviewed by

Robert J. Weir, Ph.D.
Vice President

# MUTAGENICITY EVALUATION

# OF

# L7A SYNTHETIC LAUNDRY WASTE

# FINAL REPORT

**SUBMITTED TO**

**U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND**
**WASHINGTON, D. C. 20314**

**SUBMITTED BY**

**LITTON BIONETICS, INC.**
**5516 NICHOLSON LANE**
**KENSINGTON, MARYLAND 20795**

**LBI PROJECT NO. 2683**

**AUGUST 27, 1976**

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SPONSOR: U. S. Army Medical Research and Development Command

MATERIAL: L7A Synthetic Laundry Waste

1. NAME OR CODE NUMBER: L7A Synthetic Laundry Waste
2. DATE RECEIVED: June 9, 1976
3. DESCRIPTION: White Soapy Liquid

SUBJECT: FINAL REPORT MUTAGENICITY PLATE ASSAY

# MUTAGENICITY PLATE ASSAY

## 1. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

## 2. MATERIALS

### A. Indicator Microorganisms

*Salmonella typhimurium*, strains: TA-1535, TA-98, TA-1537, TA-100, TA-1538

*Saccharomyces cerevisiae*, strain: D4

### B. Activation System (Ames et al., Mutation Research 31:347, 1975)

#### 1. Reaction Mixture

| Component | Final Concentration/ml |
|---|---|
| TPN | 4 µmoles |
| Glucose-6-Phosphate | 5 µmoles |
| Sodium Phosphate | 100 µmoles |
| $MgCl_2$ | 8 µmoles |
| KCl | 33 µmoles |
| Homogenate fraction equivalent to 25 mg of wet tissue | 0.1-0.15 ml 9,000 x g supernatant of rat liver |

#### 2. S-9 Homogenate

A 9,000 x g supernatant was prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 five days prior to kill.

## 4. SUMMARY OF PLATE TEST RESULTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: LTA SYNTHETIC LAUNDRY WASTE
B. SOLVENT: DEIONIZED WATER
C. TEST DATE: AUG. 18, 1978
NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) PER PLATE.

| TEST | SPECIES | TISSUE | HISTIDINE PLATE TA-1535 | | TA-1537 | TA-1538 | TA-98 | TA-100 | UA* |
|---|---|---|---|---|---|---|---|---|---|
| **MUTAGENICITY** | | | | | | | | | |
| SOLVENT CONTROL | --- | --- | 13 | | 28 | 15 | 57 | 126 | 90 |
| POSITIVE CONTROL** | --- | --- | > 1000 | | > 1000 | >1000 | 800 | >1000 | 357 |
| TEST COMPOUND | | | | | | | | | |
| 1.00000 UL | --- | --- | 8 | | 22 | 12 | 40* | 129 | 63 |
| 5.00000 UL | --- | --- | 10 | | 21 | 9 | 40* | 146 | 65 |
| 10.00000 UL | --- | --- | 12 | | 24 | 12 | 38* | 149 | 36 |
| 100.00000 UL | --- | --- | 10 | | 39 | 13 | 47 | 151 | 41 |
| **ACTIVATION** | | | | | | | | | |
| SOLVENT CONTROL | RAT | LIVER | 28 | 27 | 58 | 20 | 39 | 279 | 37 |
| POSITIVE CONTROL*** | RAT | LIVER | 272 | - | 493 | > 1000 | > 1000 | >1000 | 41 |
| TEST COMPOUND | | | | | | | | | |
| 1.00000 UL | RAT | LIVER | 68 | - | 44 | 29 | 41 | 232 | 36 |
| 5.00000 UL | RAT | LIVER | 37 | - | 32 | 24 | 41 | 261 | 38 |
| 10.00000 UL | RAT | LIVER | 27 | - | 34 | 23 | 33 | 317 | 37 |
| 100.00000 UL | RAT | LIVER | 70 | 29 | 41 | 22 | 38 | 310 | 37 |

* UNK. CONCENTRATIONS PER PLATE

** TA-1535 SODIUM AZIDE 10 UG/PLATE
TA-1537 9AA 10 UG/PLATE
TA-1538 4NQO 100 UG/PLATE
TA-98 4NQO 100 UG/PLATE
TA-100 SODIUM AZIDE 10 UG/PLATE
UA SODIUM AZIDE 10 UG/PLATE

*** TA-1535 ANTH 100 UG/PLATE
TA-1537 AMO 100 UG/PLATE
TA-1538 AAF 100 UG/PLATE
TA-98 AAF 100 UG/PLATE
TA-100 ANTH 100 UG/PLATE
UA UNNA 100 MICROMOLES/PLATE

## 5. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound was examined for mutagenic activity in a series of *in vitro* microbial assays employing *Salmonella* and *Saccharomyces* indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats. The following results were obtained:

### A. Toxicity

The compound was tested over a series of concentrations such that there was either quantitative or qualitative evidence of some chemically induced physiological effects at the high dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of this compound was from 1 µl to 100 µl per plate.

### B. Nonactivation Test Results

The results of the tests conducted on the compound in the absence of a metabolic system were all negative.

### C. Activation Test Results

The results of the tests conducted on the compound in the presence of the rat liver activation system were all negative. The 100 µl per plate dose with TA-1535 was repeated because of a slightly increased mutant frequency. The repeat test was negative.

### D. Conclusions

The test compound L7A Synthetic Landury Waste did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.

Submitted by:

David Brusick, Ph.D.
Director
Department of Genetics

Reviewed by:

Robert J. Weir, Ph.D.
Vice President

# MUTAGENICITY EVALUATION

# OF

# L7 SYNTHETIC LAUNDRY WASTE

# FINAL REPORT

**SUBMITTED TO**

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
WASHINGTON, D. C. 20314

**SUBMITTED BY**

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 2683

AUGUST 27, 1976

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SPONSOR: U. S. Army Medical Research and Development Command

MATERIAL: L7 Synthetic Laundry Waste

1. NAME OR CODE NUMBER: L7 Synthetic Laundry Waste
2. DATE RECEIVED: June 9, 1976
3. DESCRIPTION: Cloudy Soapy Liquid

SUBJECT: FINAL REPORT MUTAGENICITY PLATE ASSAY

# MUTAGENICITY PLATE ASSAY

## 1. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

## 2. MATERIALS

### A. Indicator Microorganisms

*Salmonella typhimurium*, strains: TA-1535, TA-98, TA-1537, TA-100, TA-1538

*Saccharomyces cerevisiae*, strain: D4

### B. Activation System (Ames *et al.*, Mutation Research 31:347, 1975)

#### 1. Reaction Mixture

| Component | Final Concentration/ml |
|---|---|
| TPN | 4 µmoles |
| Glucose-6-Phosphate | 5 µmoles |
| Sodium Phosphate | 100 µmoles |
| $MgCl_2$ | 8 µmoles |
| KCl | 33 µmoles |
| Homogenate fraction equivalent to 25 mg of wet tissue | 0.1-0.15 ml 9,000 x g supernatant of rat liver |

#### 2. S-9 Homogenate

A 9,000 x g supernatant was prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 five days prior to kill.

## 2. MATERIALS (Continued)

### C. Positive Control Chemicals

Table 1 below lists the chemicals used for positive controls in the nonactivation and activation assays.

TABLE 1

| ASSAY | CHEMICAL[a] | SOLVENT | PROBABLE MUTAGENIC SPECIFICITY |
|---|---|---|---|
| Nonactivation | Methylnitrosoguanidine (MNNG) | Water or Saline | BPS[b] |
| | 2-Nitrofluorene (NF) | Dimethylsulfoxide[c] | FS[b] |
| | Quinacrine mustard (QM) | Water or saline | FS[b] |
| Activation | 2-Anthramine (ANTH) | Dimethylsulfoxide[c] | BPS[b] |
| | 2-Acetylaminofluorene (AAF) | Dimethylsulfoxide[c] | FS[b] |
| | 8-Aminoquinoline (AMQ) | Dimethylsulfoxide[c] | FS[b] |
| | Dimethylnitrosamine (DMNA) | Saline | BPS[b] |

[a]Concentrations given in Results Section

[b]BPS = Base-pair substitution
FS = Frameshift

[c]Previously shown to be nonmutagenic

### D. Solvent

Either deionized water or dimethylsulfoxide (DMSO) was used to prepare stock solutions of solid materials. All dilutions of test materials were made in either deionized water or DMSO. The solvent employed is recorded in the Results Section.

## 3. EXPERIMENTAL DESIGN

### A. Plate Test (Overlay Method)

Approximately $10^8$ cells from an overnight culture of each indicator strain were added to separate test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For non-activation tests, the four dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests four dose levels of the test chemical were added to the appropriate tubes with cells. Just prior to pouring, an aliquot of reaction mixture (0.5 ml containing the 9,000 x g liver homogenate) was added to each of the activation overlay tubes, which were then mixed, and the contents poured over the surface of a minimal agar plate and allowed to solidify. The plates were incubated for 48 hours at 37C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation were run with each assay.

### B. Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were analyzed in a computer program and reported on a printout. The results are presented as revertants per plate for each indicator strain employed in the assay. The positive and the solvent controls are provided as reference points. Other relevant data are provided on the computer printout.

## 4. SUMMARY OF PLATE TEST RESULTS

a. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: L7 SYNTHETIC LAUNDRY WASTE
b. SOLVENT: DEIONIZED WATER
c. TEST DATE: AUG. 10, 1976
NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) PER PLATE.

| TEST | SPECIES | TISSUE | REVERTANTS PER PLATE | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | TA-1535 | TA-1537 | TA-1538 | TA-98 | TA-100 | D4* |
| **NONACTIVATION** | | | | | | | | |
| SOLVENT CONTROL | --- | --- | 13 | 28 | 15 | 57 | 126 | 50 |
| POSITIVE CONTROL** | --- | --- | > 1000 | >1000 | >1000 | 800 | > 1000 | 357 |
| TEST COMPOUND | | | | | | | | |
| 1.00000 UL | --- | --- | 14 | 23 | 10 | 58 | 118 | 41 |
| 5.00000 UL | --- | --- | 12 | 21 | 9 | 35 | 147 | 29 |
| 10.00000 UL | --- | --- | 13 | 21 | 12 | 59 | 129 | 47 |
| 100.00000 UL | --- | --- | 8 | 22 | 6 | 50 | 150 | 37 |
| **ACTIVATION** | | | | | | | | |
| SOLVENT CONTROL | RAT | LIVER | 28 | 58 | 20 | 39 | 279 | 37 |
| POSITIVE CONTROL*** | RAT | LIVER | 272 | 493 | > 1000 | > 1000 | > 1000 | 41 |
| TEST COMPOUND | | | | | | | | |
| 1.00000 UL | RAT | LIVER | 51 | 31 | 19 | 47 | 285 | 39 |
| 5.00000 UL | RAT | LIVER | 57 | 35 | 20 | 32 | 284 | 35 |
| 10.00000 UL | RAT | LIVER | 36 | 30 | 17 | 39 | 323 | 37 |
| 100.00000 UL | RAT | LIVER | 49 | 35 | 9 | 37 | 302 | 35 |

* INDICATES CONTAMINANTS PER PLATE

** TA-1535 MNNG 10 UG/PLATE
TA-1537 9AA 10 UG/PLATE
TA-1538 NF 100 UG/PLATE
TA-98 NF 100 UG/PLATE
TA-100 MNNG 10 UG/PLATE
D4 MNNG 10 UG/PLATE

*** TA-1535 ANTH 100 UG/PLATE
TA-1537 AMO 100 UG/PLATE
TA-1538 AAF 100 UG/PLATE
TA-98 AAF 100 UG/PLATE
TA-100 ANTH 100 UG/PLATE
D4 DMNA 100 MICROMOLES/PLATE

## 5. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound was examined for mutagenic activity in a series of *in vitro* microbial assays employing *Salmonella* and *Saccharomyces* indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats. The following results were obtained:

### A. Toxicity

The compound was tested over a series of concentrations such that there was either quantitative or qualitative evidence of some chemically induced physiological effects at the high dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of this compound was from 1 µl to 100 µl per plate.

### B. Nonactivation Test Results

The results of the tests conducted on the compound in the absence of a metabolic system were all negative.

### C. Activation Test Results

The results of the tests conducted on the compound in the presence of the rat liver activation system were all negative.

### D. Conclusions

The test compound L7 Synthetic Laundry Waste did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.

Submitted by:

David Brusick, Ph.D.
Director
Department of Genetics

Reviewed by:

Robert J. Weir, Ph.D.
Vice President

# MUTAGENICITY EVALUATION

# OF

# L12 SYNTHETIC LAUNDRY WASTE

## FINAL REPORT

**SUBMITTED TO**

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
WASHINGTON, D. C. 20314

**SUBMITTED BY**

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 2683

AUGUST 27, 1976

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SPONSOR: U. S. Army Medical Research and Development Command

MATERIAL: L12 Synthetic Laundry Waste

1. NAME OR CODE NUMBER: L12 Synthetic Laundry Waste
2. DATE RECEIVED: June 9, 1976
3. DESCRIPTION: Clear Soapy Liquid

SUBJECT: FINAL REPORT MUTAGENICITY PLATE ASSAY

# MUTAGENICITY PLATE ASSAY

## 1. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

## 2. MATERIALS

### A. Indicator Microorganisms

*Salmonella typhimurium*, strains: TA-1535, TA-98, TA-1537, TA-100, TA-1538

*Saccharomyces cerevisiae*, strain: D4

### B. Activation System (Ames et al., Mutation Research 31:347, 1975)

#### 1. Reaction Mixture

| Component | Final Concentration/ml |
|---|---|
| TPN | 4 µmoles |
| Glucose-6-Phosphate | 5 µmoles |
| Sodium Phosphate | 100 µmoles |
| $MgCl_2$ | 8 µmoles |
| KCl | 33 µmoles |
| Homogenate fraction equivalent to 25 mg of wet tissue | 0.1-0.15 ml 9,000 x g supernatant of rat liver |

#### 2. S-9 Homogenate

A 9,000 x g supernatant was prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 five days prior to kill.

## 2. MATERIALS (Continued)

### C. Positive Control Chemicals

Table 1 below lists the chemicals used for positive controls in the nonactivation and activation assays.

TABLE 1

| ASSAY | CHEMICAL[a] | SOLVENT | PROBABLE MUTAGENIC SPECIFICITY |
|---|---|---|---|
| Nonactivation | Methylnitrosoguanidine (MNNG) | Water or Saline | BPS[b] |
| | 2-Nitrofluorene (NF) | Dimethylsulfoxide[c] | FS[b] |
| | Quinacrine mustard (QM) | Water or saline | FS[b] |
| Activation | 2-Anthramine (ANTH) | Dimethylsulfoxide[c] | BPS[b] |
| | 2-Acetylaminofluorene (AAF) | Dimethylsulfoxide[c] | FS[b] |
| | 8-Aminoquinoline (AMQ) | Dimethylsulfoxide[c] | FS[b] |
| | Dimethylnitrosamine (DMNA) | Saline | BPS[b] |

[a]Concentrations given in Results Section

[b]BPS = Base-pair substitution
FS = Frameshift

[c]Previously shown to be nonmutagenic

### D. Solvent

Either deionized water or dimethylsulfoxide (DMSO) was used to prepare stock solutions of solid materials. All dilutions of test materials were made in either deionized water or DMSO. The solvent employed is recorded in the Results Section.

## 3. EXPERIMENTAL DESIGN

### A. Plate Test (Overlay Method)

Approximately $10^8$ cells from an overnight culture of each indicator strain were added to separate test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For non-activation tests, the four dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests four dose levels of the test chemical were added to the appropriate tubes with cells. Just prior to pouring, an aliquot of reaction mixture (0.5 ml containing the 9,000 x g liver homogenate) was added to each of the activation overlay tubes, which were then mixed, and the contents poured over the surface of a minimal agar plate and allowed to solidify. The plates were incubated for 48 hours at 37C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation were run with each assay.

### B. Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were analyzed in a computer program and reported on a printout. The results are presented as revertants per plate for each indicator strain employed in the assay. The positive and the solvent controls are provided as reference points. Other relevant data are provided on the computer printout.

4 SUMMARY OF PLATE TEST RESULTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: L12 SYNTHETIC LAUNDRY WASTE
B. SOLVENT: DEIONIZED WATER
C. TEST DATE: AUG. 10, 1976
NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) PER PLATE.

| TEST | SPECIES | TISSUE | REVERTANTS PER PLATE | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | TA-1535 | TA-1537 | TA-1538 | TA-98 | TA-100 | D4* |
| **BIOACTIVATION** | | | | | | | | |
| SOLVENT CONTROL | --- | --- | 14 | 24 | 15 | 57 | 126 | 50 |
| POSITIVE CONTROL** | --- | --- | >1000 | >1000 | >1000 | 800 | >1000 | 357 |
| TEST COMPOUND | | | | | | | | |
| 1.00000 UL | --- | --- | 12 | 18 | 15 | 39 | 128 | 41 |
| 5.00000 UL | --- | --- | 10 | 25 | 6 | 47 | 162 | 43 |
| 10.00000 UL | --- | --- | 8 | 21 | 14 | 45 | 148 | 56 |
| 100.00000 UL | --- | --- | 1 | 19 | 2 | 51 | 135 | 49 |
| **ACTIVATION** | | | | | | | | |
| SOLVENT CONTROL | RAT | LIVER | 28 | 58 | 20 | 39 | 279 | 37 |
| POSITIVE CONTROL*** | RAT | LIVER | 272 | 493 | >1000 | >1000 | >1000 | 41 |
| TEST COMPOUND | | | | | | | | |
| 1.00000 UL | RAT | LIVER | 30 | 42 | 26 | 31 | 304 | 36 |
| 5.00000 UL | RAT | LIVER | 45 | 35 | 22 | 46 | 320 | 42 |
| 10.00000 UL | RAT | LIVER | 46 | 47 | 24 | 46 | 326 | 35 |
| 100.00000 UL | RAT | LIVER | 24 | 39 | 21 | 52 | 259 | 39 |

* INV. CONVERTANTS PER PLATE

** TA-1535 SOLVENT 10 UG/PLATE
TA-1537 WATER 10 UG/PLATE
TA-1538 WATER 100 UG/PLATE
TA-98 WATER 100 UG/PLATE
TA-100 SOLVENT 10 UG/PLATE
D4 SOLVENT 10 UG/PLATE

*** TA-1535 ANTH 100 UG/PLATE
TA-1537 AMO 100 UG/PLATE
TA-1538 AAF 100 UG/PLATE
TA-98 AAF 100 UG/PLATE
TA-100 ANTH 100 UG/PLATE
D4 DMNA 100 MICROMOLES/PLATE

## 5. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound was examined for mutagenic activity in a series of *in vitro* microbial assays employing *Salmonella* and *Saccharomyces* indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats. The following results were obtained:

### A. Toxicity

The compound was tested over a series of concentrations such that there was either quantitative or qualitative evidence of some chemically induced physiological effects at the high dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of this compound was from 1 µl to 100 µl per plate.

### B. Nonactivation Test Results

The results of the tests conducted on the compound in the absence of a metabolic system were all negative.

### C. Activation Test Results

The results of the tests conducted on the compound in the presence of the rat liver activation system were all negative.

### D. Conclusions

The test compound L12 Synthetic Laundry Waste did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.

Submitted by:

David Brusick, Ph.D.
Director
Department of Genetics

Reviewed by:

Robert J. Weir, Ph.D.
Vice President

# MUTAGENICITY EVALUATION

# OF

# L14 SYNTHETIC LAUNDRY WASTE

# FINAL REPORT

**SUBMITTED TO**

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
WASHINGTON, D. C. 20314

**SUBMITTED BY**

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 2683

AUGUST 27, 1976

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SPONSOR: U. S. Army Medical Research and Development Command

MATERIAL: L14 Synthetic Laundry Waste

1. NAME OR CODE NUMBER: L14 Synthetic Laundry Waste
2. DATE RECEIVED: June 9, 1976
3. DESCRIPTION: Clear Soapy Liquid

SUBJECT: FINAL REPORT MUTAGENICITY PLATE ASSAY

# MUTAGENICITY PLATE ASSAY

## 1. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

## 2. MATERIALS

### A. Indicator Microorganisms

*Salmonella typhimurium*, strains: TA-1535, TA-98, TA-1537, TA-100, TA-1538

*Saccharomyces cerevisiae*, strain: D4

### B. Activation System (Ames *et al.*, Mutation Research 31:347, 1975)

#### 1. Reaction Mixture

| Component | Final Concentration/ml |
|---|---|
| TPN | 4 µmoles |
| Glucose-6-Phosphate | 5 µmoles |
| Sodium Phosphate | 100 µmoles |
| $MgCl_2$ | 8 µmoles |
| KCl | 33 µmoles |
| Homogenate fraction equivalent to 25 mg of wet tissue | 0.1-0.15 ml 9,000 x g supernatant of rat liver |

#### 2. S-9 Homogenate

A 9,000 x g supernatant was prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 five days prior to kill.

2. MATERIALS (Continued)

C. Positive Control Chemicals

Table 1 below lists the chemicals used for positive controls in the nonactivation and activation assays.

TABLE 1

| ASSAY | CHEMICAL[a] | SOLVENT | PROBABLE MUTAGENIC SPECIFICITY |
|---|---|---|---|
| Nonactivation | Methylnitrosoguanidine (MNNG) | Water or Saline | BPS[b] |
| | 2-Nitrofluorene (NF) | Dimethylsulfoxide[c] | FS[b] |
| | Quinacrine mustard (QM) | Water or saline | FS[b] |
| Activation | 2-Anthramine (ANTH) | Dimethylsulfoxide[c] | BPS[b] |
| | 2-Acetylaminofluorene (AAF) | Dimethylsulfoxide[c] | FS[b] |
| | 8-Aminoquinoline (AMQ) | Dimethylsulfoxide[c] | FS[b] |
| | Dimethylnitrosamine (DMNA) | Saline | BPS[b] |

[a]Concentrations given in Results Section

[b]BPS = Base-pair substitution
FS = Frameshift

[c]Previously shown to be nonmutagenic

D. Solvent

Either deionized water or dimethylsulfoxide (DMSO) was used to prepare stock solutions of solid materials. All dilutions of test materials were made in either deionized water or DMSO. The solvent employed is recorded in the Results Section.

## 3. EXPERIMENTAL DESIGN

### A. Plate Test (Overlay Method)

Approximately $10^8$ cells from an overnight culture of each indicator strain were added to separate test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For non-activation tests, the four dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests four dose levels of the test chemical were added to the appropriate tubes with cells. Just prior to pouring, an aliquot of reaction mixture (0.5 ml containing the 9,000 x g liver homogenate) was added to each of the activation overlay tubes, which were then mixed, and the contents poured over the surface of a minimal agar plate and allowed to solidify. The plates were incubated for 48 hours at 37C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation were run with each assay.

### B. Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were analyzed in a computer program and reported on a printout. The results are presented as revertants per plate for each indicator strain employed in the assay. The positive and the solvent controls are provided as reference points. Other relevant data are provided on the computer printout.

## 4. SUMMARY OF PLATE TEST RESULTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: L14 SYNTHETIC LAUNDRY WASTE
B. SOLVENT: DEIONIZED WATER
C. TEST DATE: AUG. 10, 1976
NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) PER PLATE.

| TEST | SPECIES | TISSUE | REVERTANTS PER PLATE TA-1535 | | TA-1537 | TA-1538 | TA-98 | TA-100 | D4* |
|---|---|---|---|---|---|---|---|---|---|
| **-S9 ACTIVATION** | | | | | | | | | |
| SOLVENT CONTROL | --- | --- | 14 | | 28 | 20 | 57 | 126 | 50 |
| POSITIVE CONTROL** | --- | --- | >1000 | | 206 | > 1000 | 800 | >1000 | 357 |
| TEST COMPOUND | | | | | | | | | |
| 1.00000 UL | --- | --- | 17 | | 27 | 13 | 47 | 149 | 37 |
| 5.00000 UL | --- | --- | 14 | | 18 | 4 | 52 | 172 | 35 |
| 10.00000 UL | --- | --- | 11 | | 23 | 10 | 46 | 184 | 48 |
| 100.00000 UL | --- | --- | 11 | | 13 | 15 | 53 | 165 | 46 |
| **+S9 ACTIVATION** | | | | | | | | | |
| SOLVENT CONTROL | RAT | LIVER | 28 | 11 | 53 | 20 | 39 | 279 | 37 |
| POSITIVE CONTROL*** | RAT | LIVER | 272 | | 493 | >1000 | >1000 | > 1000 | 41 |
| TEST COMPOUND | | | | | | | | | |
| 1.00000 UL | RAT | LIVER | 42 | 7 | 47 | 21 | 38 | 267 | 36 |
| 5.00000 UL | RAT | LIVER | 92 | 16 | 30 | 22 | 35 | 328 | 31 |
| 10.00000 UL | RAT | LIVER | 24 | - | 52 | 21 | 36 | 314 | 39 |
| 100.00000 UL | RAT | LIVER | 25 | - | 32 | 16 | 41 | 330 | 34 |

* INDICATES CONTAMINATED PLATE

** TA-1535 [illegible] 10 UG/PLATE
TA-1537 [illegible] 10 UG/PLATE
TA-1538 4NQO 100 UG/PLATE
TA-98 4NQO 100 UG/PLATE
TA-100 [illegible] 10 UG/PLATE
D4 [illegible] 10 UG/PLATE

*** TA-1535 ANTH 100 UG/PLATE
TA-1537 AMA 100 UG/PLATE
TA-1538 AAF 100 UG/PLATE
TA-98 AAF 100 UG/PLATE
TA-100 ANTH 100 UG/PLATE
D4 DMNA 100 MICROMOLES/PLATE

## 5. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound was examined for mutagenic activity in a series of *in vitro* microbial assays employing *Salmonella* and *Saccharomyces* indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats. The following results were obtained:

### A. Toxicity

The compound was tested over a series of concentrations such that there was either quantitative or qualitative evidence of some chemically induced physiological effects at the high dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of this compound was from 1 µl to 100 µl per plate.

### B. Nonactivation Test Results

The results of the tests conducted on the compound in the absence of a metabolic system were all negative.

### C. Activation Test Results

The results of the tests conducted on the compound in the presence of the rat liver activation system were all negative. The 1 µl and 5 µl per plate doses with TA-1535 were repeated because of slightly increased mutant frequencies. The repeat test was negative.

### D. Conclusions

The test compound L14 Synthetic Laundry Waste did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.

Submitted by:

David Brusick, Ph.D.
Director
Department of Genetics

Reviewed by:

Robert J. Weir, Ph.D.
Vice President

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